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Hemorrhagic tendency after massive transfusion of preserved blood

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Patients who receive massive blood transfusion are on the increase because of the recent progress in surgical procedures and in blood supply facilities. The hemorrhagic tendency associated with this has raised challenging questions, which have been discussed and studied for the past several years.[2, 4, 6, 9, 12, 24, 25, 28]

We have had 8 patients (5 underwent pulmonary operations and 3 underwent gastric and esophageal resections) who developed such hemorrhagic tendency in our clinic in the past 4 years. They represent 0.7 per cent of the total number of blood-receiving patients in this period.

The causes of the hemorrhagic tendency and the therapeutic problems are still far from being completely understood. In this article the results of our clinical and experimental research for the past 4 years are given.

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MATERIALS AND METHODS

In addition to our clinical experiences, experimental studies were performed with dogs and rabbits. Compatible preserved blood amounting to from 0.8 to 2.0 times the estimated circulating blood volume was infused, while the proportional amount of blood was being drawn out under general inhalation anesthesia. The blood had been prepared in 250 ml. bottles containing acid citrate dextrose solution and was stored at 4° to 6° C. for 2 or 3 weeks.

Observations were made of bleeding time, clotting time, platelet count, platelet fragility, platelet adhesiveness,[14] platelet factor III (thromboplastinogenase), platelet serotonin level,[26] and distribution of platelets after blood transfusion (by P^{32}-tagged platelets). Electromicroscopic and phase-contrast microscopic observations were made of platelets.[10] Thrombopoiesis was observed by tissue culture of the bone marrow. Determinations were also made of clot

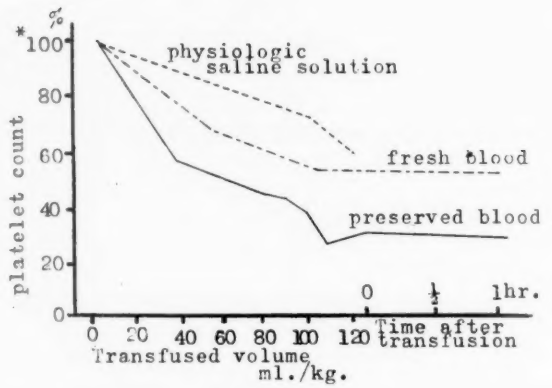

Fig. 1. Decrease in platelet count during and after transfusion (average value in per cent). A value of 100 per cent (*) corresponds to pretransfusion level.

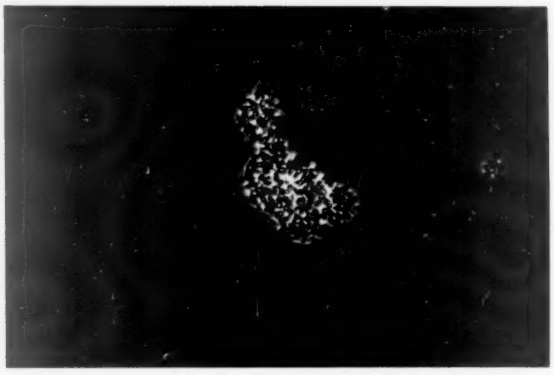

Fig. 2. Platelet clump formation.

Table I. *Platelet clump formation activity in recipient's plasma after transfusion of preserved blood (80 to 100 ml. per kilogram)*

	Before transfusion	*After transfusion*
Total No. of patients	32	32
Cases of clump formation	4	24
Per cent	12.5	75.0

retraction, recalcification time, thromboplastin generation, prothrombin (one-stage Quick method and two-stage Ware-Seegers method), fibrinogen level, fibrinolytic activity, fibrinolysin level, antifibrinolysin level, and antithrombin activity. Assays were made of heparin and thrombelastograms were taken. Capillary resistance was determined by the modified negative pressure method of Robson,[17] and serum calcium and citric acid levels[7] were also studied.

Except for several special determinations, for which separate references are given, we used methods described in the chapter on laboratory methods in *Hemorrhagic Diseases* by A. J. Quick.[16]

RESULTS AND COMMENT

Factors which cause the bleeding tendency.

Mechanism of a decrease in platelet count. The decreased platelet count which always appears after the massive transfusion of preserved blood is considered to be one of the factors which cause the bleeding tendency.[12, 24, 25] Several theories[2, 4, 5, 12, 15, 22] have been presented concerning the mechanism of this deficiency, but none of these is authoritative. In our experiments, platelets in the preserved blood show marked destruction at the third day of preservation. However, dilution with the platelet-poor plasma has a small effect on this phenomenon, and the platelet count decreases much more than can be attributed to the effect of dilution with the infusion of the preserved plasma (Fig. 1).

The appearance of platelet antibodies and agglutinins in the preserved blood is very rare, and thus cannot be the cause of the deficient platelet count which occurs in almost every case.

Studying the function of platelet formation in the bone marrow, we did not notice any abnormalities of megakaryocytes immediately or 24 hours after massive transfusion of preserved blood. Also, changes in the distribution of platelets were observed in dogs which had received the blood transfusion after reinfusion of P^{32}-tagged platelets. The platelet levels decreased markedly in the peripheral venous blood and showed a marked increase in the lung and bone marrow in comparison with the levels in control animals which received an infusion of physiologic saline solution. These facts are thought to show that the abnormal

Table II. *Morphologic platelet changes seen with an electron microscope after transfusion and incubation of preserved plasma (frequency of forms I to V)*

	I	*II*	*III*	*IV*	*V*
Before transfusion (normal)	19%	20%	38%	11%	12%
After transfusion of preserved blood, 120 ml. per kilogram	9	10	9	44	28
After incubation in preserved plasma (37° C., 30 min.)	14	10	16	37	23

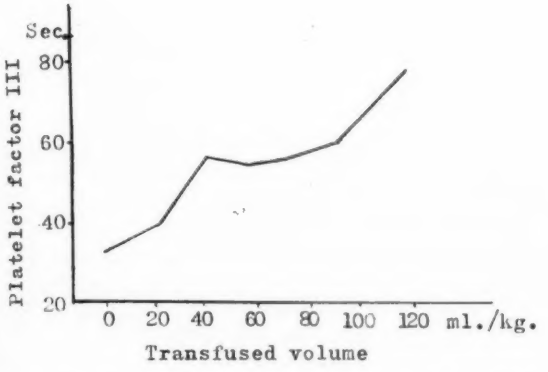

Fig. 3. Decrease of platelet factor III which occurs during the transfusion of preserved blood (average value).

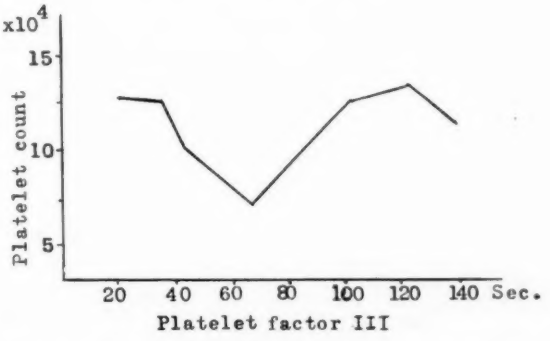

Fig. 4. Relation between platelet count and platelet factor III during transfusion of preserved blood (average value).

distribution of platelets which occurs after the blood transfusion is due to some unknown mechanism. This mechanism is still under investigation, and we will present a summary of the results which seem to be most interesting at present. When normal platelets were incubated in the compatible plasma in A-B-O system from a recipient who had received a transfusion of preserved blood, platelet clump formation appeared among the scattered platelets (Fig. 2). This phenomenon is observed in few recipients' plasma before blood is transfused (Table I). The same platelet clump formation is observed when platelets are incubated in the preserved blood. These phenomena still await further investigation, but are useful in explaining the cause of thrombocytopenia and the abnormal distribution of platelets in the body which is noted for a relatively short time after the transfusion of preserved blood.

Qualitative and morphologic platelet changes. After massive blood transfusion, in addition to the deficient platelet count, there are observed qualitative or functional changes. These include a decrease of platelet factor III (Fig. 3), which is not in proportion to the decrease of platelet count (Fig. 4); a slight increase of platelet fragility; a 20 to 50 per cent decrease of platelet adhesiveness (Fig. 5); and a decrease of the serotonin level in the platelets and of clot retraction, which is not proportional to the decrease of the platelet count (Fig. 6).

Morphologic observation with an electron microscope shows that normal platelets, in

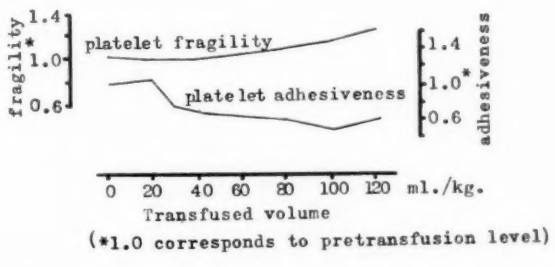

Fig. 5. Platelet fragility and adhesiveness during transfusion of preserved blood (average value).

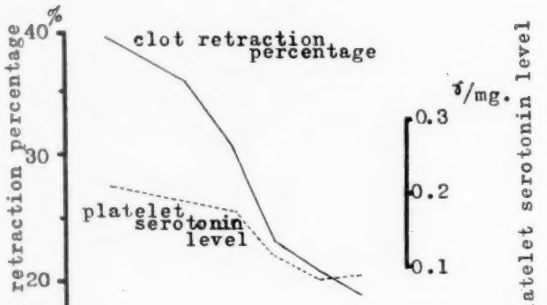

Fig. 6. Platelet serotonin level and clot retraction during transfusion of preserved blood (average value).

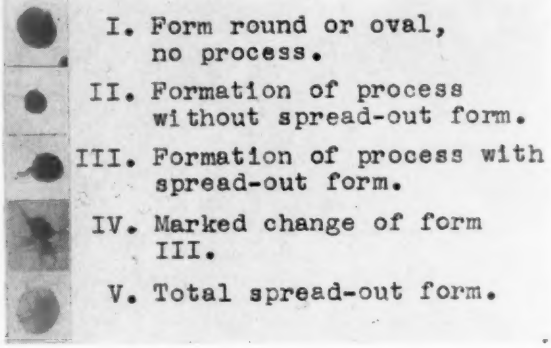
I. Form round or oval, no process.
II. Formation of process without spread-out form.
III. Formation of process with spread-out form.
IV. Marked change of form III.
V. Total spread-out form.

Fig. 7. Classification of morphologies of platelets by study with electron microscope.

most cases, have many processes and few take the spread-out form, while few platelets have processes and most of them are in the spread-out form after the preserved blood transfusion. Some ghost cells appear also. According to a platelet form classification of I, II, III, IV, and V (Fig. 7),[11] increased IV and V forms are observed after the transfusion of preserved blood (Table II). This phenomenon is very similar to that which occurs when normal platelets are incubated in preserved plasma at 37° C. for 30 minutes.

These results suggest that massive transfusion of the preserved blood causes not only a deficiency in the quantity of platelets, but also causes changes in morphology or quality.

Other factors which cause platelet disturbance. Research was carried out on other factors which are supposed to cause platelet deficiency.[27] These factors, such as operative procedures, hemorrhagic shock, and anoxia, except for fibrinolysis which will be mentioned later, were proved to have little influence on the platelet factor. When patients who received transfusion alone were compared with those who received transfusion during an operative procedure, hemorrhagic shock, or anoxia, there were no conspicuous differences in platelet deficiency (Fig. 8).

Thus, we can conclude that the transfusion of preserved blood itself is the main cause of platelet deficiency.

Vascular disturbance. In our experimental observations with an apparatus for measurement of capillary resistance of the mesentery, in most cases a decrease of capillary resistance was noted in proportion to the amount of blood transfused. However, the degree was slight, in general, and the phenomenon was not marked even in cases of accompanying oozing (Fig. 9). Other factors, such as operative procedure, hemorrhagic shock, or

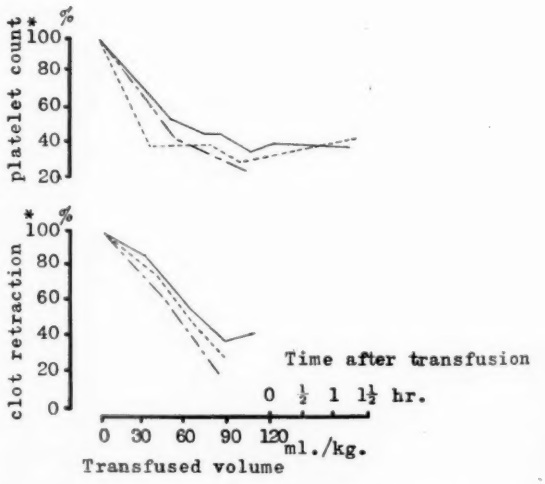

Fig. 8. Influences of operative procedure or hemorrhagic shock on platelet deficiency (average values in per cent). A value of 100 per cent (*) corresponds to pretransfusion level. Solid line, transfusion of preserved blood alone; dotted line, transfusion of preserved blood with operative procedure; and dot-dash line, transfusion of preserved blood with hemorrhagic shock.

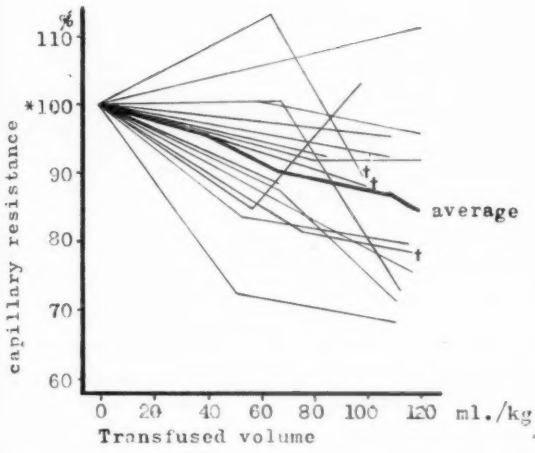

Fig. 9. Decrease of capillary resistance during transfusion of preserved blood. A value of 100 per cent (*) corresponds to pretransfusion level. Daggers indicate cases in which oozing appeared.

anoxia, have little influence on the disturbance of the vascular factors (Fig. 10). The preserved blood itself is thought to be the main cause.

Changes in the clotting mechanism. Seen most frequently after massive transfusion of preserved blood are disturbances of thromboplastin generation due to the decrease in number and qualitative deficiency of platelets, and the disturbance of thrombin generation due to decreases of prothrombin and of its conversion accelerator, especially a labile factor.[19, 28] On a thrombelastogram they are shown as prolonged r and k and decreased ma. However, these disturbances of clotting factors are not strong enough to cause bleeding as in other hemorrhagic maladies. Also, in most cases they are conspicuous about 12 hours after blood transfusion, when the deficiencies of platelet and vascular factors are already improving (Fig. 11).

Clotting factors are more strongly impaired when the massive transfusion is accompanied with other factors such as major operative procedures, hemorrhagic shock,[20] anoxia, and liver damage.[21] Liver damage initiates a vicious cycle. It causes the disturbance of clotting factors which causes more and more bleeding. Transfusion to restore the blood level in turn causes poorer liver function. Clotting factors are also strongly impaired when fibrinolysis appears.

Occurrence of fibrinolysis. Fibrinolysis can be the main cause of hemorrhagic tendency after preserved blood transfusion. We have had 4 patients with fibrinolysis, 1 of whom had gastric carcinoma and 3 of whom had pulmonary disease, out of the 8 who developed hemorrhagic disorders during operation.

Partial fibrinolysis which did not lead to the appearance of the hemorrhagic tendency was encountered relatively often, especially in pulmonary operations. The degree of fibrinolysis (total or partial) did

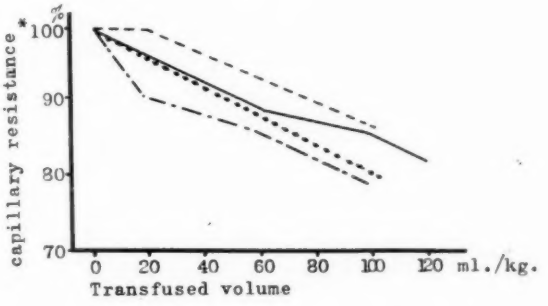

Fig. 10. Influences of operative procedure, hemorrhagic shock, or anoxia on capillary resistance (average value in per cent). A value of 100 per cent (*) corresponds to pretransfusion level. Solid line, transfusion of preserved blood alone; dotted line, transfusion of preserved blood with operative procedure; dot-dash line, transfusion of preserved blood with hemorrhagic shock; and dash line, transfusion of preserved blood with anoxia.

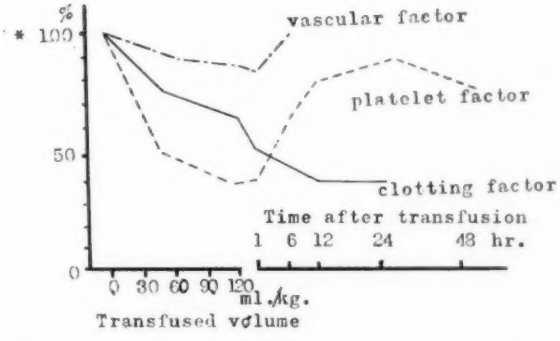

Fig. 11. Transition of the 3 factors during and after transfusion of preserved blood (average values). A value of 100 per cent (*) corresponds to pretransfusion level.

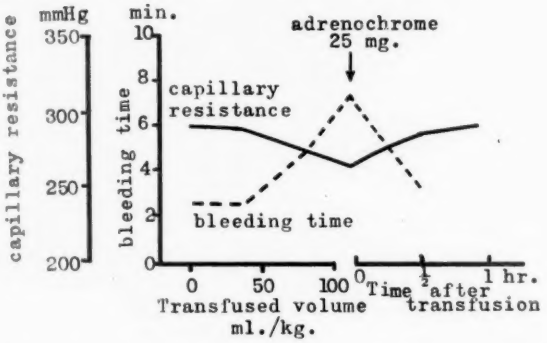

Fig. 12. Effect of intravenous administration of adrenochrome on vascular factors.

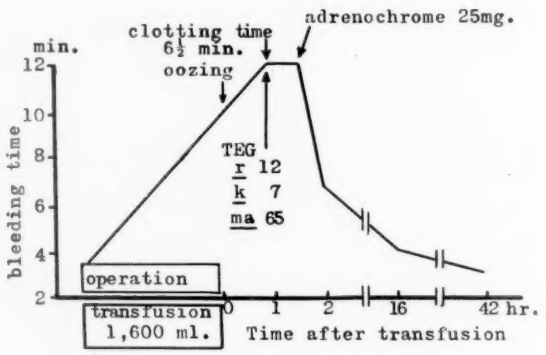

Fig. 13. Hemostatic effect of intravenous administration of adrenocrome in a clinical case (58-year-old man with stomach carcinoma).

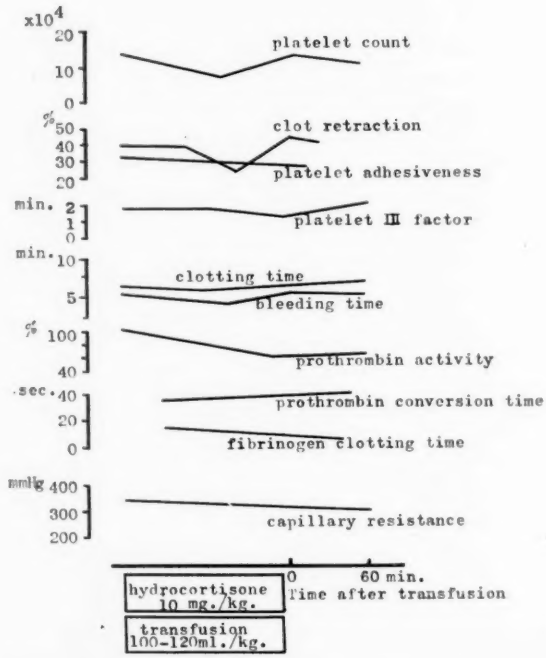

Fig. 14. Effects of intravenous drip infusion of hydrocortisone.

not correlate with the amount of transfused blood. Marked fibrinolysis was produced with operations on the lung or pancreas and in cases of hemorrhagic shock. Neither activation nor increase of fibrinolysin was noticed in the preserved blood. No decrease of the antifibrinolysin level was observed in the serum of recipients of preserved blood. Accordingly, we can impute fibrinolysis to such factors as hemorrhagic shock, surgical trauma, the specific locations of operations, and poor physical condition of patients, rather than to the preserved blood transfusion per se. Once fibrinolysis develops, it can induce hemorrhagic tendency in cooperation with many other factors involved.

Increases of circulating anticoagulants. In our experiments, neither heparin nor antithrombin levels increased in the serum of recipients of preserved blood. But, in clinical cases or in combination with major surgical trauma, these levels were proved to be much increased and probably became one of the causes of impaired coagulation.

Increased citric acid level. We recognized no remarkable decrease in the serum calcium level, despite a rapid rise of citric acid level in serum after massive blood transfusion, probably because of the release of tissue calcium into the blood.

Although the cause of a rapid drop of blood pressure followed by death, produced experimentally by citric acid intoxication, was thought to be an abnormality of electrolytes in cardiac muscle, a hemorrhagic tendency due to decreased calcium level was not produced by massive citrated blood transfusion.

Therapy and prophylaxis for the bleeding tendency. As mentioned previously, we cannot attribute the bleeding tendency after massive transfusion of preserved blood to the blood transfusion per se. The bleeding tendency varies in severity and character in each case in correlation with many other factors. Therefore, once the bleeding tendency appears, we should try to use an adequate combination of various agents which are effective for platelet, vascular, or clotting factors or fibrinolysis.

To select adequate agents, we made several observations to evaluate the effects of various agents and procedures on impaired platelet, vascular, or clotting factors in experimental cases of massive transfusion of preserved blood.

Thromboplastin preparation. Concerning platelet factors, thromboplastin preparation does improve somewhat the action of thromboplastinogenase or act as its substitute. It also shortens clotting time when thromboplastin generation is impaired. Thromboplastin preparation also increases capillary resistance approximately 40 per cent and shortens bleeding time.

Adrenochrome. Adrenochrome is effective in cases of impaired vascular factors (Fig. 12). It causes a hemostatic effect which strengthens resistance of the vascular wall. It proved useful in a case of bleeding time prolonged for over 10 minutes during operation (Fig. 13).

Intravenous drip infusion of hydrocortisone. There have been many opinions on the effectiveness of hydrocortisone.[3, 12, 18] In fact, there is a considerable variation in its effectiveness, depending upon amount and method of its administration. For example, our experiments show that hydrocortisone is more effective when it is used in continuous drip administration, all through the duration of blood transfusion, than when the same dose is administered only during the first half or latter half of the blood transfusion or after it.

When 10 mg. per kilogram of hydrocortisone is administered continuously by intravenous drip throughout massive transfusion of preserved blood, it can markedly reduce impairment of platelet, clotting, and vascular factors (Fig. 14). Thus, it reduces bleeding and decreases the amount of blood to be transfused.

ACTH and DOCA.[3] When 1 U. per kilogram of ACTH or 0.5 mg. per kilogram of DOCA is given before blood transfusion, it decreases platelet deficiency but has little effect on prothrombin activity or vascular factors.

Platelet transfusion.[8, 23] Platelet transfusion decreased the impairment of platelet factors, that is, it increases platelet count and improves platelet function (Fig. 15). It improves thromboplastin generation impaired by the transfusion of preserved blood

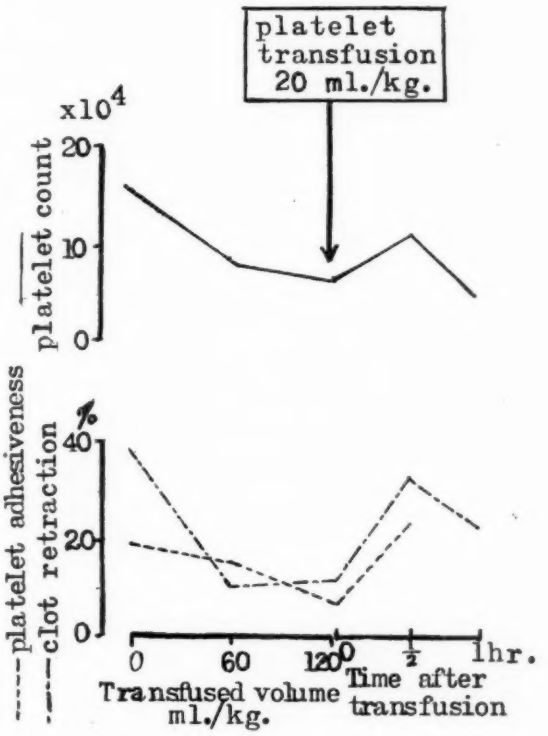

Fig. 15. Effect of platelet transfusion on platelet factor impaired by massive transfusion of preserved blood.

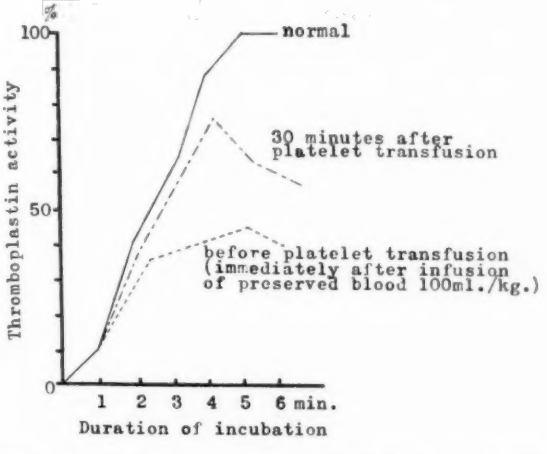

Fig. 16. Effect of platelet transfusion on thromboplastin generation impaired by massive transfusion of preserved blood. (Thromboplastin generation test: normal serum plus normal plasma plus recipient's platelet suspension.)

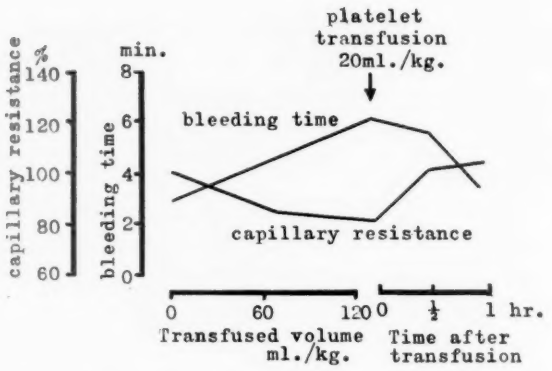

Fig. 17. Effect of platelet transfusion on vascular factors impaired by massive transfusion of preserved blood.

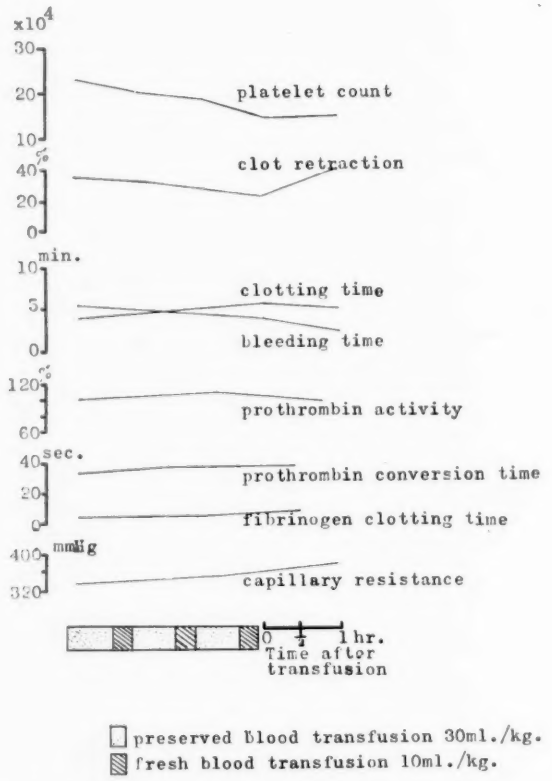

Fig. 18. Transition of various deficiencies when fresh blood is given alternately with preserved blood.

(Fig. 16) and is also effective for vascular factors (Fig. 17). However, these effects are all transient, and we have practical difficulties in collecting platelets and repeating their administration easily enough to meet an emergency need, although we have plenty of time with chronic bleeding diseases.

Fresh blood transfusion.[1, 4] When fresh blood was given with nonwettable instruments after transfusion of preserved blood, we noticed increases of platelet count, of platelet factor III, of platelet adhesiveness, and of clot retraction. Fresh blood transfusion had a good effect on clotting factors. It improved thromboplastin generation and prothrombin activity, and also strengthened capillary resistance. These effects, however, were not marked.

When fresh blood was given alternately with preserved blood, for example, 10 ml. per kilogram of fresh blood given alternately with 30 ml. per kilogram of preserved blood, it was more effective in controlling impairment of platelet, clotting, and vascular factors, and worked better in preventing a bleeding tendency than when the same total amount of fresh blood was given at one time (Fig. 18).

Therapeutic agents for fibrinolysis. The most effective agents for fibrinolysis are hydrocortisone given intravenously,[13] ε-aminocaproic acid given intravenously, or a combination of both. In our experiments, we had many cases in which fibrinolysis was quickly controlled with the intravenous administration of hydrocortisone or ε-aminocaproic acid (Fig. 19).

In our clinical experience, also, hydrocortisone, ε-aminocaproic acid, or a combination was successful in suppressing fibrinolysis in pulmonary and other operations.

CONCLUSION

Causes of the bleeding tendency after massive transfusion of preserved blood. Bleeding tendency cannot be caused by a single factor. Combinations of deficiency in number and function of platelets and impaired vascular factors caused by massive transfusion of preserved blood brought about hemorrhage in combination with local factors such as physical stimulation. Clotting disturbance, promoted by other factors such as hemorrhagic shock, anoxia, poor physical condition, major surgical trauma, and fibrinolysis, prolongs bleeding and leads to uncontrolled bleeding, that is, the bleeding tendency.

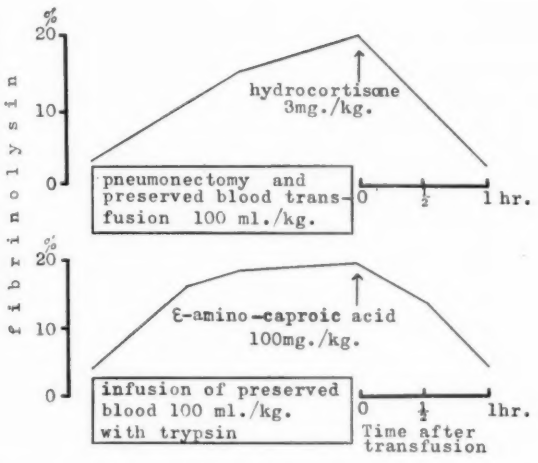

Fig. 19. Correction of fibrinolysis by intravenous administration of hydrocortisone or ε-aminocaproic acid.

Therapy and prophylaxis for the bleeding tendency after massive transfusion of preserved blood. In our experience, it was impossible in most cases to determine the causative factor with screening tests at the moment of difficulty. For this reason, we needed various kinds and large doses of drugs to control the bleeding tendency which had already developed. Nevertheless, we cannot always expect complete success.

In our opinion, the emphasis should be on prevention. When it is expected that a massive blood transfusion will be given especially when causal factors such as hemorrhagic shock, anoxia, liver damage, and so forth are present, we should always take complete prophylactic precautions as early as possible.

Our methods include (1) administration of ε-aminocaproic acid for prevention of fibrinolysis, (2) continuous intravenous drip instillation of hydrocortisone, (3) alternating transfusions of fresh and preserved blood, and (4) administration of an adequate combination of adrenochrome, thromboplastin preparations, and calcium. Also, close attention should be paid to the factors which impair the coagulation system.

SUMMARY

The bleeding tendency after massive transfusion of preserved blood is caused by intrinsic and extrinsic factors such as hemorrhagic shock, anoxia, liver damage, and so forth, rather than the blood transfused per se. The best approach to this problem is prevention and the methods described were found to be effective.

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5-Hydroxytryptamine and antidiuresis after operation

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Despite intensive research, some facets of postoperative renal function still lack proper understanding. Current concepts of renal disorders after trauma have focused attention on the importance of glomerular filtration,[13] and the earlier view of tubular dysfunction as the primary cause of traumatic renal failure may have to be revised.[25] The changes in the electrolyte balance after operation are in a rather good correlation with the known actions of adrenocortical hormones, especially aldosterone, and with the behavior of body cells as osmotic regulators. However, water retention in excess of electrolytes, although the renal function may otherwise be normal, is one of the subtle features of the postoperative renal function.[31]

Besides renal pathologic changes, humoral agents, especially the antidiuretic hormone (ADH), have received attention. Postoperatively urine possesses antidiuretic activity which indubitably is in relation to an increased ADH secretion.[8] The role of ADH in postoperative oliguria is also confirmed by observations in patients with diabetes insipidus,[31] but the common occurrence of a persistent oliguric phase shows that ADH is not the complete solution of the problem.

Platelets, the most fragile cellular blood elements, are known to contain 5-hydroxytryptamine (5-HT, serotonin),[16, 32] a substance with antidiuretic properties. Previously it has been suggested that the 5-HT released from disintegrated platelets during extracorporeal circulation[24] has a certain influence on the renal function during cardiopulmonary bypass operations.[11] The present study was made in an attempt to clarify whether or not 5-HT bears any relationship to postoperative water retention.

MATERIAL AND METHODS

Thirteen patients, aged 32 to 61 years, who underwent intrathoracic operations without a heart-lung machine, were studied. The daily urine output was measured pre- and postoperatively with an accuracy of 5 ml. The postoperative measurement was begun the moment the patient was returned to bed from the operating room. The urinary excretion of 5-hydroxyindolacetic acid (5-HIAA),* the main metabolic derivative of 5-HT, was determined spectrophotometrically[28] on 2 consecutive preoperative days and 7 times after the operation over a postoperative period of 10 days. The platelet count and hematocrit were determined by routine techniques. In 5 patients the 5-HT of whole blood was determined after acetone extraction[3] with the rat stomach preparation[29] on 2 preoperative days and 5 times after the operation. The recoveries of 5-HT added to blood have been approximately 40 per cent. No values have been adjusted for rate of recovery.

Siliconized glassware and needles† were

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*5-HIAA was supplied by The Upjohn Company, Kalamazoo, Mich.

†Desicoute, Beckman Instruments, Inc., Fullerton, Calif.

Table I. *5-HT and 5-HIAA values and other data on main collection days (mean and range)*

	Preoperative days		*Postoperative days*				
	2	*1*	*1*	*2*	*4*	*6*	*9*
Platelets ($\times 10^3$/ml.)	335 220-470	345 231-500	271 39-490	304 205-450	346 239-490	353 233-487	346 232-411
5-HT (ng.*/ml.)	47 24-110	34 17-60	45 13-106	29 12-80	32 20-40	37 32-45	30 12-60
5-HIAA (mg./24 hr.)	3.7 1.9-9.3	3.0 1.9-7.6	5.4 2.5-9.0	4.3 2.2-9.3	4.1 2.2-7.7	3.9 2.1-8.9	4.0 1.5-7.2
Hematocrit (vol. %)	46 41-49	43 36-47	45 43-50	46 44-48	44 35-50	38 33-42	40 34-50
Urine output (ml./24 hr.)	1,141 715-1,575	1,144 435-2,010	644 210-1,200	512 155-675	657 250-950	874 720-1,220	910 450-1,355
Specific gravity (+ 1,000)	20 15-26	20 14-27	30 26-35	29 24-35	28 23-33	25 15-30	19 5-29

*Nanogram equals $10^{-3}\mu g$.

used throughout the study. The urine and blood-acetone samples were kept at –20° C. before determinations. Reserpine compounds, chlorpromazine, and banana feeding were avoided. The usual surgical regime with premedications and transfusions was not changed.

RESULTS

The principal findings are recorded in Table I. Increase of the specific gravity paralleled the familiar restriction of the urine output. The daily urine flow reached approximately the preoperative level on the ninth day after operation. In the mean values of 5-HT, no clear-cut decrease became evident. The mean values, however, do not show the whole truth, since the individual values of 5-HT followed the platelet count, in which the drop was equally distributed to the first and second postoperative days. For example, in 1 patient the platelets decreased from a preoperative level of 257,000 to 39,000 per milliliter on the first day after operation and the 5-HT from 30 to 13 ng.* per milliliter, respectively. In one other illustrative case the changes in platelets were from 360,000 to 277,000 per milliliter on the second postoperative day and changes in the 5-HT were from 46 to 12 ng. per milliliter, respectively. If there was no noteworthy change in the platelet count, the 5-HT values did not show a decreasing trend. Fig. 1 demonstrates the amounts of 5-HIAA excreted and the urine output in the periods in question. The significance of the mean differences of the 5-HIAA excretion was analyzed by use of the t test. The mean of the 2 preoperative values of each patient was used as the control figure. There was a significant increase in the excretion on the first and second postoperative days ($p < 0.01$) and also on the fourth day ($p < 0.02$). Under basal conditions the 5-HIAA excretion remains rather constant in the same individual.[28] The variation from day to day in our laboratories has not, as a rule, exceeded 20 per cent. The 5-HIAA values necessitate, however, an interpretation against this range. When it is taken into account, only the values on the first 2 postoperative days exceeded the preoperative level. This occurred in 7 patients, whereas in 6 patients the excretion was unaffected by the operation. The hematocrit for the most part remained unchanged.

*Nanogram equals 10^{-3} μg.

DISCUSSION

The response of the platelet count to a major operation is usually an initial depression followed by an elevation to preoperative

levels and above. During the second postoperative week the platelet count may be 2 or 3 times the preoperative level.[17] This elevation has been of particular interest, since it coincides with the peak occurrence of thrombotic complications. The initial depression, which can reach considerable dimensions by even 2 hours after the operation,[19] is also associated with a shortened bleeding time.[30] It is due, at least in part, to an increased platelet conglutination, and to disintegration of platelets in the operative field and local hemorrhages. This local factor is nicely illustrated by the observation that the drop can be prevented by rinsing the wound edges with heparin and epinephrine.[19]

When the initial decrease in the platelet count occurred in the present series it was associated with a decrease in the whole blood 5-HT and an elevation in the urinary excretion of 5-HIAA. Theoretically, the blood transfusions given to the patients can be held responsible for the changes observed. However, stored blood, unless specially handled, contains only a few viable platelets[12] and it has been demonstrated that 5-HT is oxidized in human serum.[20]

In a study of the value of 5-HIAA excretion as an indication of impending thrombotic complications after operation (including open-heart operations) in a group of patients, the 5-HIAA values were not elevated during the first postoperative days.[27] This difference from the present findings is probably due to the amount of renal suppression. The decreased glomerular filtration rate and renal blood flow usually recover after operation, but this might be delayed after cardiopulmonary bypass operations. The finding of a decreased 5-HIAA excretion during operation is in accord with other reports.[11, 24] The excretion of 5-HIAA is due to an active tubular excretion[7, 21] and is uninfluenced by the urinary pH.[21] According to these concepts the urinary 5-HIAA ought to be independent of the urine flow per se, but it is certainly affected by a decreased renal blood flow and renal pathologic changes. In such states, as has been pointed out earlier,[11] 5-HIAA values may give an unreliable impression unless the 5-HT values of the blood are measured simultaneously.

The rise in the specific gravity after operation indicates that we are not dealing with postoperative renal failure. Admittedly, no significant renal pathologic changes can be suspected to exist in the present series.

The antidiuretic effect of 5-HT has been demonstrated in all the usual laboratory animals[1, 5, 9, 10, 23, 26] and in man.[4, 14, 15] Divergent opinions exist on the mode of this action. Perfusion experiments have shown a strong vasoconstrictor response in the renal vessels,[6, 22] but several studies with renal clearance measurements have presented evidence that the antidiuretic action of 5-HT is due to an increased water reabsorption from the tubules.[23, 26] The finding that 5-HT contracts the ureteral muscle[2] makes a decision no easier. An intact posterior lobe of the hypophysis is not essential in 5-HT antidiuresis[1, 5] and 5-HT antidiuresis has also been demonstrated in patients with diabetes insipidus.[4] This pathway, however, must be taken into account because it has been observed that 5-HT decreases the amount of neurosecretory substance in the posterior pituitary gland.[18]

In view of the data given, suggestive evidence is found that 5-HT, a substance with antidiuretic properties, is released into plasma after major operations. The question

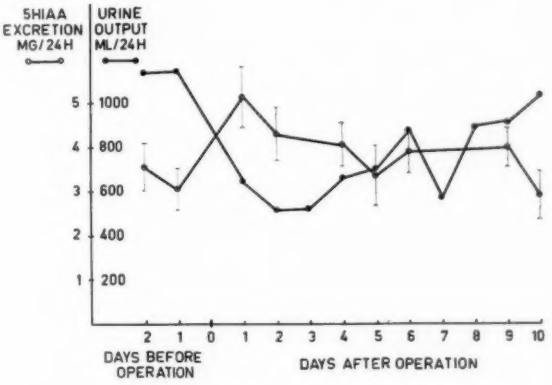

Fig. 1. The mean urine output and excretion of 5-hydroxyindolacetic acid (± standard error of the mean) of 13 patients before and after operation.

of the extent of its responsibility for the antidiuresis cannot be answered. Restriction of the postoperative urine output occurred also in patients without evidence of 5-HT release. The present findings confirm, however, the assumption that humoral vasoconstrictors play a part in the pathogenesis of posttraumatic renal function.[25]

SUMMARY

Thirteen patients were studied after major operations to find out whether or not the typical pattern of the postoperative platelet count was associated with alterations in the amount of 5-hydroxytryptamine in the blood. It was found that in patients with a drop in the platelet count on the first 2 postoperative days the 5-hydroxytryptamine content of whole blood was correspondingly decreased, and a simultaneous increase occurred in the urinary excretion of 5-hydroxyindolacetic acid. In view of the antidiuretic properties of 5-hydroxytryptamine, it is suggested that this humoral agent participates in postoperative antidiuresis.

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Intravenous l-norepinephrine as a cause of reduced plasma volume

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Ten years ago, intravenous l-norepinephrine was first shown by Goldenberg and co-workers[6] to be of value in the clinical management of certain hypotensive states. Since that time, the drug has been found useful in a variety of surgical and medical emergencies. It has become apparent, however, that sudden withdrawal of the drug after its prolonged administration is frequently accompanied by an immediate fall of the arterial blood pressure to such low levels as to endanger the life of the patient. It is common clinical practice to decrease the rate of administration of l-norepinephrine slowly and progressively in an effort to prevent the occurrence of this phenomenon. Incidental observations in the course of other studies on shock[8] suggested that patients who receive l-norepinephrine show a steadily decreasing plasma volume. The purpose of the present investigation was to determine the effect of the intravenous administration of l-norepinephrine on plasma volume in dogs. During the course of this study, a method was evolved to permit instantaneous discontinuation of the drug without the occurrence of the commonly observed secondary hypotension.

The investigations of von Euler,[11, 12] Holtz,[7] and others evaluated the physiologic and biochemical properties of l-norepinephrine and established its significance as a vasopressor agent. It was not until 1949, when clinical application of the drug began, that reports appeared regarding complications which occurred with its use. The majority of these publications stressed the necrotizing effect of the drug on the tissues at the site of injection.[3] Early case reports show that sudden withdrawal of l-norepinephrine may result in severe hypotension and shock. It has, therefore, been recommended that the rate of administration be reduced gradually.[9] Saave[10] reported 3 instances. In one, the patient died after unintentional sudden discontinuation of l-norepinephrine and in the other two, withdrawal of the drug was accompanied by a steady fall of arterial blood pressure and a deterioration of the patients' condition. After adequate blood transfusions both of the patients recovered.

This paper will present a physiologic explanation for the secondary hypotension described in the literature and encountered by the authors in the clinical use of l-norepinephrine.

METHODS

Thirty-four healthy fasting mongrel dogs which ranged from 10.0 to 20.3 kilograms in body weight were employed for these studies. All animals were splenectomized at least 3 days prior to the experiment because of the well-recognized lability of splenic blood volume in the dog. Anesthesia was induced by the intravenous administration

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of pentobarbital sodium, 30 mg. per kilogram of body weight. One femoral artery was cannulated and connected to a strain gauge manometer for continuous recording of arterial blood pressure. The other femoral artery was exposed to facilitate repeated puncture for sampling of arterial blood. One femoral vein was cannulated for the intravenous administration of a continuous drip of l-norepinephrine.* The contralateral femoral vein was used for the injection of dye for blood volume determination.

The blue dye method with acetone extraction, as modified by Chinard,[2] was adopted for the blood volume studies. A sample of arterial blood was drawn to provide a plasma blank for the spectrophotometer. An accurately weighed amount of Evans blue dye† was injected intravenously. After an interval of 10 minutes, an arterial sample was withdrawn. The concentration of the dye was determined in plasma with the use of a Beckman spectrophotometer at a wave length of 620 μ employing cuvettes with a 10 mm. layer. The blank plasma sample obtained before the injection of the dye was first read against distilled water. After conversion of percentage transmission into values for optical density, the plasma volume was calculated. Hematocrit was determined by centrifugation of a specimen for 20 minutes at 3,000 r.p.m. and was corrected for plasma trapping by a factor of 0.96. Blood volume was calculated by the formula:

$$BV = \frac{PV}{100\text{-}H \times F \text{ cells}} \times 100$$

where BV is blood volume, PV is plasma volume, and H is corrected hematocrit. F cells is a factor of 0.88 to correct peripheral to total body hematocrit.

Procedure. An initial determination of blood pressure and blood volume was made. Immediately thereafter, a continuous infusion of l-norepinephrine in the amount of 4.0 mcg. per kilogram of body weight per minute was begun. The infusion was continued for a period of 100 minutes. Ninety minutes after the beginning of the l-norepinephrine administration, a second sample of arterial blood was drawn, and Evan's blue dye injected. At 100 minutes, the final plasma volume determination was carried out and the l-norepinephrine discontinued.

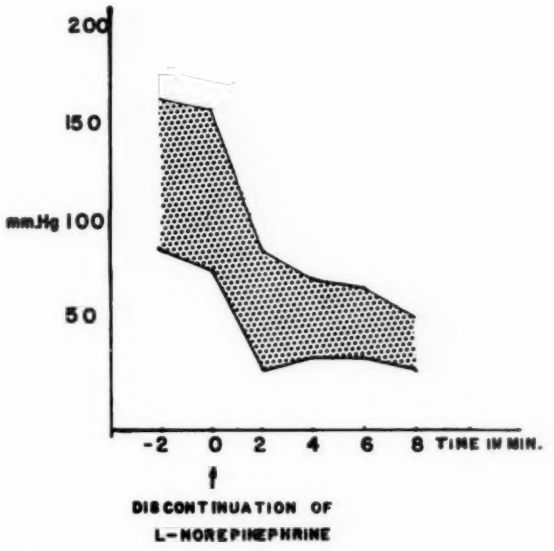

Fig. 1. Mean change in arterial blood pressure in 8 animals after discontinuation of l-norepinephrine is illustrated. Severe hypotension results from the administration of the drug to these normal anesthetized animals.

In a group of 10 animals, arterial blood pressure was monitored continuously before, during, and after a 100-minute period of l-norepinephrine administration (4 mcg. per kilogram of body weight per minute). Eight minutes after discontinuation of the drug, blood pressure fell from the hypertensive levels maintained by l-norepinephrine to a mean of 54/28 mm. Hg (Fig. 1). Two animals in this group showed a progressive diminution in arterial blood pressure and died within 2 hours after completion of the experiment. An illustration of this severe hypotensive response in a single dog is given in Fig. 2.

In pilot experiments on a preliminary series of 8 animals, an average decrease in plasma volume of 29.3 ± 12.5 per cent was noted. However, in these animals a volume of 400 to 800 ml. of saline solution was employed as a vehicle in the administration of

*Levophed, Winthrop Laboratories, New York 18, N. Y.
†Supplied through the courtesy of Eli Lilly & Company, Indianapolis, Ind.

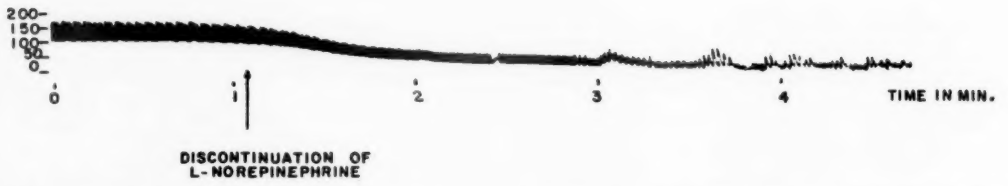

Fig. 2. Severe hypotension occurs in a normal anesthetized dog on withdrawal of l-norepinephrine after a 100-minute infusion at 4 mcg. per kilogram per minute.

l-norepinephrine. The hematocrit of these animals decreased by an average of 4.6 per cent. It was apparent that the large volume of saline solution administered may have had a physiologic effect independent of the effect of the drug.

In another series of 8 animals, the experimental protocol was modified by elimination of the large amounts of saline solution which were used as a vehicle for the l-norepinephrine. In each experiment, the total dose of the drug was carried in 30 ml. of normal saline solution. Changes in plasma volume in this series of animals are presented in Table I. It is noted that there was a mean decrease in plasma volume of 26.8 ± 18.5 per cent. Associated with this decrease was a mean increase in hematocrit of 4.2 per cent. Calculated total blood volume diminished on an average of 21.2 ± 23.0 per cent.

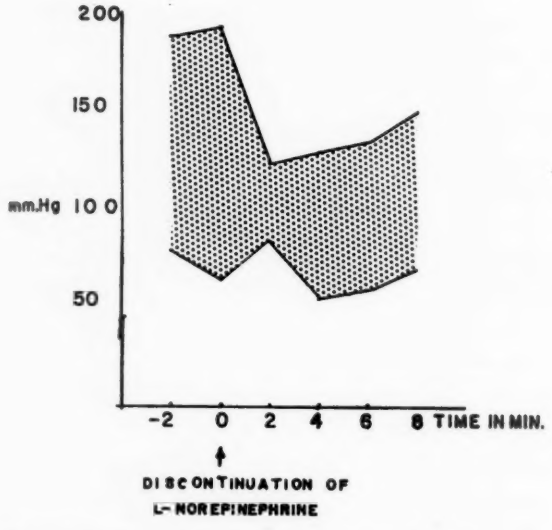

Fig. 3. When the l-norepinephrine infusion was discontinued in these 8 animals, an amount of dextran equal to 15 per cent of estimated blood volume was rapidly infused intravenously. In contrast to results illustrated in Fig. 2, the arterial blood pressure was maintained at normal levels in these animals.

All animals showed an elevation in arterial pressure with the beginning of the l-norepinephrine infusion. At the end of the infusion, a volume of dextran equal to 15 per cent of estimated blood volume was infused intravenously within 2 to 3 minutes. In contrast to the previously described animals (Fig. 1), these animals did not develop a secondary hypotension. Blood pressure returned from the hypertensive levels maintained by the drug to normal and remained there (Figs. 3 and 4).

Control experiments. Six animals were employed in several types of control experiment. When the infusion did not contain l-norepinephrine, there was no significant change in either plasma volume or hematocrit (Table I). Since l-norepinephrine had been demonstrated to produce hypovolemia in normal dogs, it was decided to test the drug in the presence of shock due to hemorrhage. Although the drug elevated arterial blood pressure in the presence of hemorrhagic shock, it caused an average loss of plasma volume of 18.8 per cent in addition to that already lost by hemorrhage.

These control experiments were interpreted as indicating that factors in the protocol other than the l-norepinephrine did not produce the observed changes in plasma volume. They also indicate that l-norepinephrine causes the same loss in plasma volume in dogs in hemorrhagic shock as it does in normal anesthetized dogs.

DISCUSSION

These experiments on the effect of intravenous l-norepinephrine in the normal dog show a striking diminution in plasma vol-

ume. The mechanism of this decrease in plasma volume is not definitively demonstrated in this study. A possible explanation may lie in the diminished volume of the peripheral vascular bed caused by the intense vasoconstriction. Such vasoconstriction might be postulated to "squeeze" fluid from the intravascular to the extravascular space. Some support of this theory is seen in the changes that were noted in hematocrit. The hemoconcentration that occurred during the experimental infusion (Table I) does suggest that fluid is leaving the intravascular compartment. The well-recognized discrepancy in peripheral hematocrit and total body hematocrit makes it difficult to correlate quantitatively the loss of plasma and the rise in hematocrit. Calculation of red blood cell volume changes from the data in Table I shows a mean decrease of 13.1 per cent. However, this does not represent a statistically significant change from the volume present before l-norepinephrine administration.

It was clearly demonstrated that in animals in which severe hypotension occurred on discontinuation of the drug, normal arterial pressure could be restored by the rapid infusion of dextran or whole blood. This is presumptive evidence that in at least some clinical situations, the hypotension seen after discontinuation of l-norepinephrine may be due to the drug itself, rather than to the underlying disease for which the drug was originally administered.

This experimental study is not completely analogous to the clinical situation, since each of the animals had a relatively normal cardiovascular status before the administration of the drug. It can only be presumed that the hypovolemia-inducing action of l-norepinephrine demonstrated in normal animals would be present also in clinical situations in which the drug is used. It was demonstrated that hypovolemia due to hemorrhage could be further aggravated by the intravenous administration of l-norepinephrine. Despite the fact that blood pressure was maintained at more normal levels after hemorrhage, administration of the drug caused a further diminution of plasma volume.

The results of this study have been applied clinically in a limited number of patients. Because of the emergency nature of situations which require the intravenous administration of l-norepinephrine, it has not been possible to obtain blood volume measurements under controlled conditions in patients, despite several attempts. During the course of this study, there were 6 surgical patients in whom l-norepinephrine was employed as a temporary measure to maintain arterial pressure and in whom attempts to reduce the rate of administration of the drug in a stepwise fashion resulted in progressive hypotension. In each patient, the effort to discontinue the drug was made only after it was considered that the underlying cause of the patient's initial hypotension had been corrected. In these 6 patients, it was possible to discontinue instantaneously the administration of l-norepinephrine by the rapid intravenous injection (3 to 5 minutes) of from 300 to 500 ml. of blood or dextran.

It should be pointed out that in clinical practice the volume of blood or plasma expander infused is less than that demonstrated to be lost in the present experimental study. Smaller volumes have been found adequate

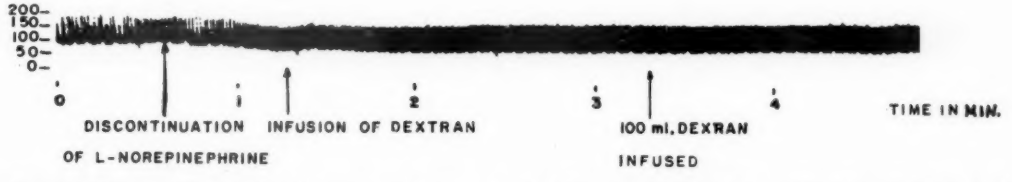

Fig. 4. When arterial blood pressure began to decline 1 minute after cessation of l-norepinephrine infusion, dextran infusion restored blood volume and prevented hypotension.

Table I. *Changes in plasma volume, blood volume, and hematocrit after l-norepinephrine infusion*

Experiment No.	*Weight*	*Plasma volume*			*Blood volume*			*Hematocrit*		
		Before	*After*	*% change*	*Before*	*After*	*% change*	*Before*	*After*	*% change*
1	18.3	950	588	-38	1,610	1,030	-36	46.1	48.0	+1.9
2	17.0	956	924	- 3	1,630	1,700	+ 4	46.1	51.8	+5.7
3	18.0	1,128	745	-34	1,950	1,330	-32	48.0	49.8	+1.8
4	20.3	1,445	772	-47	2,220	1,230	-45	39.8	42.2	+2.4
5	17.0	812	661	-19	1,440	1,210	-16	49.9	51.8	+1.9
6	12.0	657	644	- 2	1,120	1,280	+14	47.0	56.6	+9.6
7	12.5	465	232	-50	930	478	-49	56.7	58.5	+1.8
8	13.0	547	433	-21	910	822	-10	45.2	53.8	+8.6
Average				-26.8 ± 18.5 (S.D.)*			-21.2 ± 23.0 (S.D.)*			+4.2 ± 3.2 (S.D.)*
Controls										
1	17.5	517	508	- 2	1,050	1,030	- 2	56	56	0
2	9.7	464	462	0	1,080	1,050	- 3	49	50	+1
Average				- 1.0			- 2.5			+0.5

*A statistically significant change.

and appear to reduce the danger of hypervolemia. Many clinical situations have been encountered in which this rapid infusion would not permit the discontinuation of l-norepinephrine. In these circumstances, it is considered that the hypotension is due not to the hypovolemia caused by l-norepinephrine, but rather to the underlying circulatory disorder which originally necessitated the administration of the drug.

The findings of this investigation are not surprising if one considers the fact that l-norepinephrine is, in its properties and effects, closely related to epinephrine. In dog experiments, Bainbridge and Trevan[1] found that sudden discontinuation of epinephrine infused intravenously resulted in shock. Similar observations are recorded by Freeman and associates who demonstrated that the intravenous administration of epinephrine resulted in hypovolemia. The latter investigators found an average decrease in plasma volume of 30.6 per cent after a 90-minute infusion of epinephrine, a result which is strikingly similar to the results of the present study with l-norepinephrine. They found evidence that at least part of the fluid loss with epinephrine is into the lumen of the intestinal tract.

It is interesting to recall the history of epinephrine which, after its discovery, was heralded as a panacea for shock. Within a short time, it was proved to be contraindicated in the treatment of this condition. Epinephrine itself produces hypovolemic shock. The initial enthusiasm which many physicians had for l-norepinephrine waned when it was realized how few patients survive its administration. The present study demonstrates that l-norepinephrine, like epinephrine, produces hypovolemic shock. We do not wish to take a position regarding the relative indications for, and merits of, epinephrine and l-norepinephrine. Rather, we wish to point out the interesting historical and physiologic parallel between the two drugs for thoughtful consideration.

SUMMARY

A study of the effects of l-norepinephrine on arterial pressure and plasma volume has been carried out in a series of 34 anesthetized, splenectomized dogs.

1. After sudden discontinuation of the drug, a decline in arterial blood pressure to hypotensive levels may occur. This observation confirms clinical experience.

2. The intravenous administration of l-norepinephrine (4 mcg. per kilogram of body weight per minute) for a period of 100 minutes in a series of 8 dogs resulted in an average decrease in plasma volume of 26.8 per cent.

3. These data demonstrate that the hypo-

tension encountered with the sudden discontinuation of l-norepinephrine may be due to hypovolemia caused by the drug. This secondary hypotension may be prevented by rapid infusion of blood or dextran (15 per cent of estimated blood volume) immediately after withdrawal of l-norepinephrine. Clinical confirmation of this observation has been obtained.

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Treatment of malignant retroperitoneal sarcomas with use of the second-look approach

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The most effective treatment for primary retroperitoneal tumors is surgical excision. Obviously, the earlier the diagnosis is made and operation carried out, the better the results. Unfortunately, since most of the sarcomas are slow-growing tumors, they are quite large at the time of their initial finding. The recurrences of these tumors are also slow growing and often reach enormous size before the recurrence is suspected and operative treatment is again undertaken. It is a well-known fact, however, that soft-tissue tumors which occur on the extremities can be treated effectively and for a long period of time with multiple excisions as the recurrences are noted.

In 1949, because of the disparity in accomplishment in lymph-node positive and lymph-node negative patients, the idea of performing reoperation on the lymph-node positive patients with cancer of the stomach, colon, or rectum from 4 to 9 months after the original procedure was suggested.[2] The second operation was carried out at a time when the patient was asymptomatic. It has been found that approximately 50 per cent of the lymph-node positive cancer patients still harbored residual tumor at the occasion of the first re-entry of the abdomen. Some successes with this method suggested the possibility that the second-look procedure might be employed to increase the number of survivors in patients with large retroperitoneal sarcomas in which the cure rate, at least in our experience, was negligible. In fact, the records of this hospital failed to show any cures in patients operated upon for sarcomatous retroperitoneal tumors prior to employment of second-look procedures.

Our plan of treatment of retroperitoneal sarcoma with the second-look approach is essentially the same as that for the visceral cancers. Because of the slow growth of most of these tumors, the interval between "looks" had been lengthened to 10 to 18 months. In addition, some of the reoperations have been carried out because of signs and symptoms of recurrence.

RESULTS

To date 8 patients have been treated according to the second-look principle (Table I) and of these 8 patients, 2 are alive and well over 5 years since the original procedure. Two patients are living and well 7 and 8 years, respectively, since the primary procedures, although the last look in both cases was positive. There is 1 patient who died 11 years after the original operation and 1 year after his last look, which had been negative. One patient died of the sarcoma after 11 years and the other 2 patients died of their disease in less than 5 years.

CASE REPORTS

Case 1. On March 22, 1948, Mrs. N. H., a 63-year-old woman had an excision of a large

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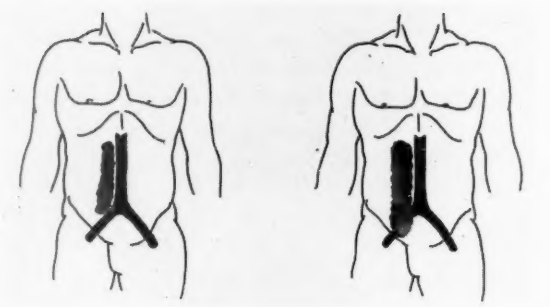
rhabdomyosarcoma which involved the left ovary and retroperitoneal space. In addition, the tumor was intimately adherent to the sigmoid colon and a loop of ileum. The uterus and left tube and ovary were excised but no intestine was removed. Postoperatively, deep x-ray therapy was given to the area. Eight months later, on Nov. 8, 1948, the patient returned to the hospital with small bowel obstruction due to a residual tumor mass located at the ileocecal junction and involving the tissues at the level of the bifurcation of the inferior vena cava. The terminal ileum and cecum were excised, along with the neoplasm, after which deep x-ray treatment again was given. Another exploratory operation was performed on July 15, 1949, at a time when the patient was

Table I

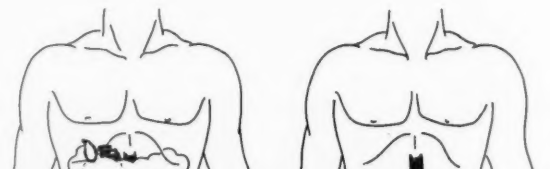
Patient	*Age at onset*	*Sex*	*Type of sarcoma*	*Original operation*	*Status at reoperations and date**	*Present status*	*Length of survival after original procedure (yr.)*
N. H.	63	F	Rhabdomyosarcoma	Excision of mass with adherent left ovary, tube, and overus (1948)	Positive, 1948; negative, 1949	Living and well	12
G. C.	54	M	Fibrosarcoma	Excision of original retroperitoneal tumor (1950)	Positive, 1953; positive, 1953; positive, 1953; positive, 1953; negative, 1953	Living and well	10
R. E.	18	M	Myxochondrosarcoma	Subtotal excision of tumor involving right ilium (1953)	Hemipelvectomy, 1954; positive, 1954; positive, 1955; positive, 1955; positive, 1956; negative, excision of recurrence in 1959 and again in 1960	Living	7
E. N.	47	M	Liposarcoma	Removal of tumor from left retroperitoneal area and left nephrectomy (1952)	Positive, 1955; negative, 1956; positive, 1957; positive, 1959	Living and well	8
E. H.	42	F	Myxosarcoma	Excision presacral myxosarcoma (1947)	Positive, 1954; positive, 1955	Dead of sarcoma	11
M. S.	63	M	Fibrosarcoma	Excision of mass from left perirenal area (1947)	Positive, 1956; negative, 1957	Dead, no sarcoma at autopsy	11
I. Z.	64	F	Leiomyosarcoma	Excision of large tumor from pelvis (1955)	Positive, 1957; positive, 1957	Dead of sarcoma	4
J. P.	53	M	Fibrosarcoma	Removal of 6 Kg. mass along with the right kidney	Positive, 1958; positive, 1958	Dead postoperatively without recurrence	1

*As of Oct. 1, 1960.



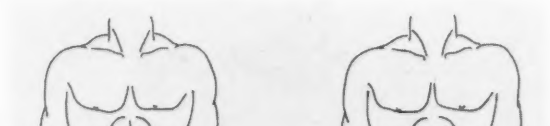
A *B*

Fig. 1. Case 2. Schematic representation of first 2 procedures performed at another hospital. *A*, First operation, July, 1950 (excision of retroperitoneal fibrosarcoma). *B*, Second operation, February, 1953 (partial excision of symptomatic residual tumor).



A *B*

Fig. 2. Case 2. First 2 operations performed at University of Minnesota Hospitals. *A*, Third operation, May, 1953 (excision of all gross residual tumor). *B*, Fourth operation, December, 1953 (second look).



A *B*

Fig. 3. Case 2. Eventual attainment of a cancer-free state at sixth operation. *A*, Fifth operation, June, 1954 (third look). *B*, Sixth operation, June, 1955 (fourth look, negative).

completely asymptomatic and without clinical evidence of recurrence. At this time, no residual tumor was found. This patient was last seen in August, 1960, 12 years, 5 months, after the original procedure, and 11 years, 1 month, after the last negative look. At this time, she has no clinical signs of residual tumor and appears completely well. The last look was negative.

Case 2. On Aug. 3, 1950, Mr. G. C., a 54-year-old white man, underwent an exploratory laparotomy, at which time a large retroperitoneal sarcoma was removed. The patient remained well until March, 1953, when an enlarging abdominal mass was again noted along with urinary symptoms. Subtotal resection of the mass was carried out and the patient was then referred here for more definitive therapy. At the time of his first admission to this hospital, he had evidence of local recurrence of a tumor, but no evidence of distant spread. On May 13, 1953, the patient underwent excision of a huge fibrosarcoma (6.4 Kg.) which extended from the level of the gall bladder and liver to the deep pelvis. The entire colon and 75 cm. of the terminal ileum were removed, along with the tumor. Intestinal continuity was established by ileoproctostomy.

On Dec. 16, 1953, a second-look procedure was carried out while the patient was asymptomatic. Two small nodules of recurrence at the lateral aspect of the right common iliac artery and over the second sacral vertebra were found and removed. The patient remained well and on June 28, 1954, another exploratory procedure was carried out. At this time, 1 lymph node medial to the right common iliac vein was positive, although many biopsies gave negative results. On June 2, 1955, the patient underwent exploratory operation and no recurrence was found in the 16 specimens of tissue removed for histologic examination. The patient, when last seen in August, 1960, was completely free of symptoms and without clinical evidence or recurrence. He does have persistent diarrhea because of extensive loss of ileum and colon.

Case 3. On. Oct. 15, 1953, R. E., an 18-year-old man, underwent palliative excision of a myxochondrosarcoma which involved the inner aspect of the right ileum. Eight months later, the right femoral nerve became paralyzed by massive tumor recurrence which involved the right wing of the ileum and right pelvis. At this time, he was referred to the University of Minnesota Medical Center. No evidence of pulmonary metastasis was observed on roentgenographic examination. On May 24, 1954, a right hemipelvectomy was performed. Postoperatively, he did well and 5 months later, on Oct. 26, 1954, during an asymptomatic phase, a second-look operation was performed. At this time, 3 fairly well circumscribed masses of tumor were present. One was located in the pelvis,

fixed between the tissues of the rectum and the base of the bladder; another was adherent to the descending colon; and a third mass was encountered at the inferior margin of the right kidney. All these nodules were excised, without sacrifice of any organs.

Five months later, on April 5, 1955, when the patient was without evidence of clinical recurrence, a third look was performed. At this time, the only evidence of tumor was limited to two 3 cm. nodules to the right of the urinary bladder. These were excised without sacrifice of the organ. Eight months later, in December, 1955, a fourth look was carried out at a time when the patient was asymptomatic. Two small tumor nodules were found opposite the second and third lumbar vertebrae and these were removed. In addition, a number of additional biopsy specimens were taken, all of which were negative. Finally, on May 31, 1956, during an asymptomatic period, the patient underwent a fifth look and no evidence of cancer was found grossly. Two biopsies of suspicious areas yielded negative histologic results.

The patient remained well until October, 1959, when he developed a swelling at the hemipelvectomy site. At a sixth look he was found to have a recurrence arising from the amputated ilium which intimately involved the mesocolon on the right side of the abdomen. The ilium was re-resected and the right colon and its mesentery were excised with re-establishment of intestinal continuity. The patient has returned since with recurrence in the right ilium treated by complete removal of the remaining ilium.

Case 4. On October 27, 1952, Mr. E. N., 47-year-old man, had excision of a liposarcoma of the left retroperitoneal area along with a left nephrectomy. He remained well until 3 years later, when a left lower quadrant mass was noted. The patient was referred to the University of Minnesota Medical Center for excision of the mass. On Nov. 8, 1955, at a second exploratory operation, a 4.5 Kg. mass of recurrent liposarcoma was removed. On Aug. 7, 1956, the patient was clinically well and without evidence of recurrence. At that time, a third look failed to reveal any recurrent cancer.

However, in June of 1957, the patient returned with a large left upper quadrant mass and partial large bowel obstruction. On June 21, 1957, an 8 cm. tumor of the left upper quadrant, along with 2 smaller masses located on the left diaphragm and several smaller tumors in the retropancreatic area were removed. They proved to be liposarcoma. Postoperatively, irradiation was given to the patient. He remained well until May, 1959, when he returned because of a mobile right upper quadrant mass. An intravenous pyelogram showed an upward displacement of the right kidney. At operation on May 27, 1959, a 10 by 15 by 20 cm. recurrent mass arising from the perirenal fat was excised. In addition, 2 other suspicious areas, one in the anterior abdominal wall of the left upper quadrant and the other in the sigmoid mesocolon, were removed. These were negative for malignancy on microscopic study. The patient continues to be well without evidence of recurrence and has returned to work. The last look was positive.

Case 5. Mrs. E. H., a 42-year-old woman, had undergone excision of a presacral myxosarcoma in 1947. She was then well for 5½ years, and then the abdomen began to enlarge markedly. She was first seen here in December, 1954, at which time a recurrence of the mass was obvious. Roentgenographic examination failed to show any evidence of distant metastases and on Dec. 24, 1954, a 3 Kg. tumor was excised, along with a portion of the terminal ileum and colon.

The patient remained well until August, 1955, when a left suprapubic mass was noted. Exploratory operation on Aug. 25, 1955, confirmed the presence of a large 8 cm. tumor mass adherent to the bladder, abdominal wall, and the small bowel in several areas. In addition, there were 7 smaller tumor nodules attached to the small bowel and 5 similar nodules on the liver surface, none of which invaded more than 1 cm. in depth. All these nodules were excised along with a 62 cm. segment of ileum which was densely adherent to the main tumor mass. The patient refused the proposal of an additional look procedure in March, 1956. On Feb. 4, 1958, the patient died after a steadily worsening course from residual sarcoma.

Case 6. Mr. M. S., a 63-year-old man, was operated upon in 1947 for a left upper quadrant tumor which was partially excised. The pathologic diagnosis at that time was hypernephroma. Irradiation of the area followed the operation and the patient was without medical aid until July, 1956, when he noted an abdominal mass and left upper quadrant discomfort associated with anorexia. The patient then saw his own physician, who advised a second course of irradiation, and he was then referred to the University of Minnesota Medical Center.

On physical examination, there was a large, hard, fixed left abdominal mass. Laboratory tests at that time showed a slight anemia and a small left pleural effusion with no function of the left kidney on intravenous pyelogram, although the right appeared to function normally. On review of the slides originally taken, the opinion of the pathologist was that the lesion was a fibrosarcoma. Prior to operation a tap of the pleural effusion was negative for tumor cells.

On Aug. 14, 1956, the patient again underwent exploratory operation and a 10 Kg. retroperitoneal fibrosarcoma was excised along with

the left kidney, left colon, a portion of the duodenum, and the left adrenal gland. A paravertebral and periaortic node dissection was also performed.

He was then followed closely and had gained 20 pounds and felt well when he was admitted early in 1957 for a second look. On Feb. 8, 1957, the patient again underwent exploratory operation and no tumor was found although 9 biopsy specimens were taken. The patient was last seen here 8 months after the operation (October, 1957) at which time he was feeling well and had gained some weight. A chest roentgenogram taken at that time was negative for metastases. The patient died suddenly on Jan. 10, 1958, and autopsy failed to disclose the cause of death. No residual tumor was found.

Case 7. On Jan. 6, 1955, Mrs. I. Z., a 64-year-old woman, sought medical advice with regard to an obvious pelvic tumor. A 13 by 14 by 12.5 cm. mass was removed from the deep pelvis and a pathologic diagnosis of a leiomyosarcoma was made. The patient remained well until March, 1957, when a mass in the upper abdomen again was noted. Physical examination at this admission showed the upper abdominal mass and also a recurrence of the pelvic mass. Consequently, on April 8, 1957, the pelvic mass was excised and the woman was well until August, 1957, when a right upper quadrant mass was noted. On Sept. 18, 1957, a 30 by 20 by 9 cm. tumor attached to the right lobe of the liver was excised along with a portion of the liver. A smaller right lower quadrant tumor was also removed. The patient was given cobalt therapy postoperatively. She did well, was gaining weight, and was admitted for a second look in the fall of 1958. On physical examination, a right upper quadrant mass was found. Chest roentgenogram and planigrams showed bilateral pulmonary metastases. The patient was not operated upon. On March 22, 1959, the patient died from the leiomyosarcoma.

Case 8. On Oct. 23, 1957, J. P., 53-year-old man, noted right upper quadrant pain and an abdominal mass of relatively short duration. On Nov. 6, 1957, a large fibrosarcoma which weighed 6 Kg., involved the edge and inferior surface of the liver, surrounded the right kidney, involved the adventitia of the aorta and iliac vessels, and extended into the pelvis, was removed. The patient remained well until 3 weeks prior to a second admission on Feb. 10, 1958, at which time an enlarging right upper quadrant mass was palpated. Excision was performed and the patient received cobalt radiotherapy postoperatively. Five months later, the patient was re-admitted with right upper quadrant pain and bilateral lower extremity edema. While in hospital the patient became jaundiced; partial bowel obstruction at the level of the duodenum also was present. On July 18, 1958, exploratory operation was carried out and massive recurrence throughout the abdomen was found. The larger of these recurrences was removed in order to relieve the obstruction of the upper jejunum. The patient's jaundice relented somewhat postoperatively, but *Candida albicans* septicemia supervened and the patient died with residual neoplasm Aug. 7, 1958.

DISCUSSION

Pack and his associates[1] state that retroperitoneal tumors are famous for their propensity to recur after radical excision. In many of the studies of retroperitoneal tumors in the literature, the average 5-year survival rate is less than 10 per cent and the 10-year survival rate is even more discouraging. It was the poor results in this hospital with these neoplasms that persuaded one of us (O. H. W.) to implement the second-look procedure in these cases.

The general plan of treatment has been the same as with the second-look procedure for abdominal visceral cancers. The one exception has been that since the sarcomatous lesions are slow growing, the interval between operations has been longer, ranging from 12 to 18 months. Also, because of the fact that large tumors in this area are often resectable even when symptomatic, there have been a number of so-called second-looks performed at a time when the patient had obvious recurrent disease.

Fourteen of the 25 repeat exploratory procedures performed in the 8 cases reported were undertaken during a symptomatic phase. However, only patients who underwent such procedures when asymptomatic are living and well today! In fact, two of these patients have been declared "negative" at a final look and are without clinical evidence of disease more than 10 years since the original operation. One hospital death occurred after operation in this group of patients, a mortality rate of 12.5 per cent by patient and 4 per cent by operation.

SUMMARY

The experience with use of the second-look program for large retroperitoneal sarcomas suggests definitely that this method of

treatment has merit in the surgical attack upon such tumors. The high salvage rate obtained is heartening and warrants the attention of the profession.

ADDENDUM

Since this report was written, 2 patients (Cases 3 and 4) have undergone additional operations. The patient in Case 3 was operated upon on March 1, 1961, and recurrent tumor in the right ilium was removed as well as a segment of involved small bowel. He was examined in June, 1961, at which time he was well and without evidence of recurrence. The patient in Case 4 returned in late October, 1960, with bowel obstruction. Re-exploration of the abdomen on Nov. 4, 1960, showed far-advanced disease which was the cause of the obstruction. He died on Dec. 18, 1960, after discharge from the hospital.

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Paper electrophoresis of extracts from normal and malignant human breast tissue

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Electrophoresis of the serum in patients with cancer has been carried out by many workers and no specific changes in the protein spectrum have been found. There is usually a diminished amount of albumin and an alteration in the alpha component of the globulin fraction, but these are both changes that are found in other diseases marked by debilitation and tissue breakdown.

The protein content of both normal and malignant tissues has been studied in animals and human beings, but there have been practically no reports on the electrophoretic pattern of proteins extracted from human malignant tissue. This paper will describe a method to show that there is a difference in protein structure between normal and malignant tissue in at least 1 human organ.

Barry,[2] working with rat fibrosarcoma extracts, produced electrophoretic patterns which were reproducible from one preparation to another. Miller and associates[4] demonstrated marked differences in the electrophoretic pattern between normal muscle and rhabdomyosarcoma in mice. Abrams and Cohen,[1] working with normal human tonsillar tissue, produced patterns from the tissue extract which had some similarities to those of human serum.

MATERIALS AND METHODS

The normal and malignant tissues were obtained from specimens that had been removed surgically. The tissue was stored at +4° C. until it was analyzed. The analysis was always performed on the day the specimen was removed from the patient. One gram of carcinoma or 1 Gm. of normal breast tissue from which as much fat as possible was removed was cut into small pieces with scissors and washed twice with 0.89 per cent saline solution to remove as much blood and lymph as possible. The tissue was then mixed with fine sand (sand flour, 325 mesh) and 3 ml. of 0.89 per cent saline solution and was ground with a mortar and pestle. The resulting mixture was then centrifuged at a relative centrifugal force of 620 for 20 minutes, and the supernatant fluid was submitted to electrophoretic analysis with Beckman's Spinco apparatus. A Veronal buffer of pH 8.6 and ionic strength 0.075 was used; a sample of 0.05 ml. was applied to the paper strip and a constant current of 2.5 Ma. was connected for 16 hours. The paper strips were then stained by the alcoholic bromphenol blue method and scanned by an Analytrol.

RESULTS

Thirteen portions of carcinoma of the breast and 13 portions of normal breast tissue have been studied. In 11 cases the carcinoma and the sample of normal tissue were obtained from the same patient. In the malignant tissue extract the average albumin content according to electrophoretic analysis was 19.62 per cent compared to 80.38 per cent globulin. In the normal breast tissue the albumin content was 51.82 per cent

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compared to 48.28 per cent globulin (Table I). The globulin portion was divided into fractions with similar mobilities to serum globulin fractions and there was found to be no great difference in the proportions of the globulin fractions between the malignant and normal tissues (Table II). It was possible to estimate the percentages in only 8 of the 13 normal portions of tissue, as there was no distinct dividing mark between the various fractions in the other 5. Alpha$_1$ and alpha$_2$ were estimated together, as any division of the two would have been arbitrary.

DISCUSSION

By the use of paper electrophoresis it has been shown that there is approximately 4 times as much globulin as albumin in malignant breast tissue, and approximately equal proportions in normal breast tissue. Other

Table I. *The percentage of albumin and globulin in malignant and normal breast tissue*

Case No.	Age (yr.)	Histologic grading of tumor	Carcinoma		Normal tissue	
			Albumin	Globulin	Albumin	Globulin
1	48	Well differentiated	18.8	81.7	51.5	48.5
2	52	Average	15.7	84.3	52.6	47.4
3	44	Average	3.6	96.4	50.4	49.6
4	56	Average	21.3	78.7	59.1	40.9
5	32	Undifferentiated	23.3	76.7	64.5	35.5
6	71	Well differentiated	32.6	67.4	46.2	53.8
7	71	Undifferentiated	26.2	73.8	—	—
8	58	Average	21.4	78.6	47.3	52.7
9	64	Average	11.5	88.5	66.7	33.3
10	77	Well differentiated	33.3	66.7	50.0	50.0
11	79	Average	17.7	82.3	46.2	53.8
12	53	Average	15.1	74.9	—	—
13	58	Average	14.5	85.5	41.5	58.5
14	21	—	—	—	42.5	57.5
15	—	—	—	—	55.2	44.8
		Mean	19.6	80.4	51.8	48.2

Table II. *The percentage of the different globulins in malignant and normal breast tissue*

Case No.	Carcinoma			Normal tissue		
	Alpha$_1$ and alpha$_2$	Beta	Gamma	Alpha$_1$ and alpha$_2$	Beta	Gamma
1	24.3	21.1	54.6	25.0	25.0	50.0
2	22.7	22.7	54.6	—	—	—
3	18.2	39.5	42.3	32.2	25.4	42.4
4	24.6	22.2	53.2	37.2	23.3	39.5
5	23.2	23.2	54.6	—	—	—
6	20.7	22.4	56.9	27.8	30.6	41.6
7	34.2	25.0	40.8	—	—	—
8	15.2	54.5	30.3	24.1	34.5	41.4
9	25.9	33.4	40.7	—	—	—
10	23.8	35.7	40.5	28.5	34.3	37.2
11	26.2	22.6	55.2	—	—	—
12	22.2	33.0	44.8	—	—	—
13	30.0	28.3	41.7	—	—	—
14	—	—	—	22.0	28.0	50.0
15	—	—	—	34.6	19.2	46.2
Mean	24.1	33.4	42.5	27.5	31.3	41.2

normal and malignant tissues, including stomach and colon, were also studied, but no such significant differences were found. Both the normal and the malignant tissues gave an electrophoretic pattern which tended to resemble the pattern of malignant breast tissue. This suggests that human tumor extracts may have similar electrophoretic patterns, but that normal tissues show considerable variation, as has been shown by Greenstein[3] in animals. Degree of histologic differentiation did not appear to exert any influence on the type of electrophoretic pattern.

CONCLUSIONS

By the use of paper electrophoresis it has been shown that there is 4 times as much globulin as albumin in human breast carcinomas, whereas normal breast tissue contains approximately equal proportions of the two proteins. However, the relative amounts of the various globulin fractions in both the malignant and normal breast tissue are not significantly different.

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Observations on the isolated ileal loop as a substitute in urinary tract operations

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The advent of radical pelvic surgery has, in the past decade or more, been an impetus to urologists to find newer and better methods of preserving kidney function. We are now truly living in the age of spare parts. Two years ago, we presented a series of experiments which illustrated total replacement of the ureter by a loop of ileum.[3] A dog with a solitary kidney and an ileal ureter remained in good health with normal electrolyte balance for 1 year. Fig. 1 is an actual photograph of a solitary kidney, ileal ureter, and normal dog bladder in one of our animals.

Our original idea was conceived as a possible method of total replacement of the ureter in children with adynamic ureters. Swenson has used approximately the same procedure, but decreased the size of the ileum by resecting the antimesenteric portion of the ileum throughout its length to reduce the absorptive surface. He advised its use only in unilateral cases when repeated tests had proved that there was no peristalsis. All patients had been repeatedly checked before this procedure was embarked upon. No patients with bilateral disease would be treated until those with unilateral disease had been observed for several years. He favored and performed a one-stage procedure.[4] It could be a valuable method in cases in which ureteropyeloplasty has failed. Postoperative strictures would be less likely to occur because of the similarity in size of the ileum and pelvis.

The practical application of this procedure, slightly modified, is shown in Fig. 2. This is a drawing of the kidney in a young girl, 21 years of age, with a solitary adynamic hydronephrotic kidney. Two attempts to reconstruct the pelviureteral junction had failed. Pyuria was constant and uncontrollable, in spite of all forms of medication. We believed that the persistent pyuria was due to pooling of the infected urine in the lower dilated calyx.

A loop of ileum 12 inches long was anastomosed to the lower calyx. This was done retroperitoneally behind the ascending colon. The distal end of the ileal loop was anastomosed end-to-side to the bladder. It is now 3 years since this procedure was carried out. The urine is negative except for an occasional pus cell. The electrolyte determinations taken on June 17 were blood urea nitrogen, 16.8 mg. per cent; serum chlorides, 100 mEq. per liter; serum potassium, 3.92 mEq. per liter; and serum sodium, 142 mEq. per liter. The patient is in exceedingly good health.[1]

Partial replacement must not be forgotten. It is an excellent procedure for replacing the lower part of the ureter that has been destroyed or damaged after radical pelvic op-

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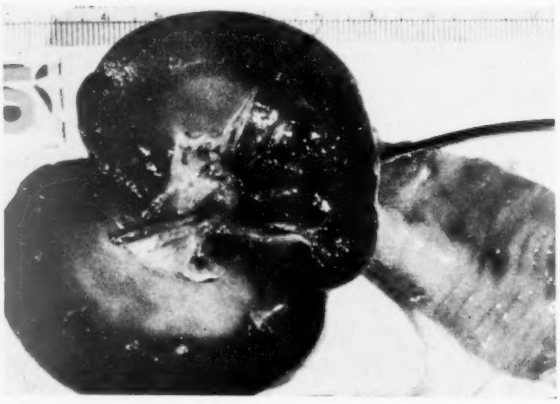

Fig. 1. The solitary kidney from one of our animals has been bisected and the ileal ureter opened. Note the normal appearance of the kidney and ureter. A probe has been passed from the ileal ureter into the kidney pelvis.

Fig. 2. Drawing of solitary adynamic hydronephrotic kidney of 21-year-old girl. Dependant drainage has been established by a calycoileoneocystostomy. The ureter was left as a safety valve. Before the procedure, pyuria had been constant.

erations, trauma from stones, inflammatory lesions, and obstruction due to fibrosis after radiation. Fig. 3 illustrates a case in which the lower end of the ureter was partially constricted after irradiation for carcinoma of the cervix. Hydronephrosis was present. A loop of ileum 6 inches long was inserted between the distal end of the ureter and the bladder. It is interesting to note that 18 months were required for this kidney to completely recover its original condition.

Bricker[2] and many others have used a loop of ileum to replace the bladder. The distal end of the ileal loop is brought out through the abdominal wall as a urinary fistula. This necessitates the wearing of some form of receptacle to catch the urine. We conceived the idea of inserting the distal end of the ileal loop into the sigmoid, thus eliminating the external urinary fistula. The peristaltic action of the ileum might protect the kidney against ascending infection, which occurs so frequently, when the ureters are transplanted directly into the sigmoid. If ascending infection were prevented, the electrolyte balance might still be a problem because of the increase in absorptive area due to the use of the sigmoid for drainage. We were unaware of the work of Turnbull and Higgins[5] at the Cleveland Clinic, a report of which was published just prior to the time we began our study.

We conducted a series of experiments on dogs to test the feasibility of the procedure. Our approach to the subject changed as we proceeded with each experiment. Five experiments were performed.

METHODS

Experiment 1. In a one-stage procedure, a loop of ileum 6 inches long was isolated. The proximal end was closed. A valve was fashioned at the distal end and inserted into the sigmoid, just above the peritoneal reflection, after the method of Turnbull. Both ureters were transplanted into the ileum. All animals died within 4 days. Three deaths were considered to be due to anesthesia. The remaining 5 dogs died with symptoms suggestive of uremia. No gross pathologic lesion

was found at postmortem examination. Histologic studies showed a protein exudate into Bowman's capsule and the proximal tubules.

A staged procedure seemed advisable. In the original procedure of Turnbull and Higgins[5] the ileal bladder was decompressed temporarily by a Foley catheter inserted into the proximal end of the ileal loop. In our first experiment, edema in the valve due to operative trauma and possibly mild constriction at the junction with the sigmoid may have in reality formed a closed loop. If the ileal loop were formed as a first step and time allowed for edema to subside and proper drainage of the loop into the sigmoid to become established, the outcome might be different.

Experiment 2. In a three-stage procedure, an isolated loop of ileum was formed as in Experiment 1, and inserted into the sigmoid. After 1 month, as a minimum, the right ureter was transplanted into the ileal loop. The loop was observed to be draining well. The animals did well and had no fever. No electrolyte imbalance occurred. Intravenous pyelograms were normal. The question arose as to whether the animals would survive if we performed a left nephrectomy. This would make them comparable to our animals which lived with one kidney and an ileal ureter. In our original experiment, the urine drained into the bladder, while in the latter the urine drained into the sigmoid. A left nephrectomy was performed in 2 dogs as a third stage.

In our first dog, the blood urea level rose to 240 mg. per cent in 7 days and 270 mg. per cent in 10 days. The animal gradually developed loss of appetite, muscular twitching, nausea, vomiting, and loss of weight. It died on the tenth day. The second dog followed an identical course. The electrolytes otherwise remained normal. Postmortem examination showed no gross pathologic lesion. Fig. 4 is a postmortem photograph of the solitary kidney which was taken from the second dog. Histologic studies of the solitary kidney from both animals showed only a protein exudate into Bowman's capsule (Fig. 5). In the tubules there was also a protein exudate and well-marked vacuolation (Fig. 6).

Experiment 3. In a three-stage procedure, an isolated ileal loop was formed and in-

Fig. 3. An intravenous pyelogram, 18 months after replacement of the lower third of the ureter with an isolated loop of ileum. Thirty minutes after injection of Skiodan the patient voided and film was taken. The ileal ureter is faintly visualized. Note excellent cupping in minor calyces.

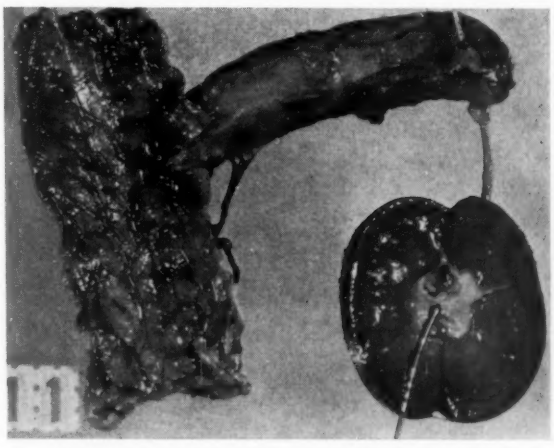

Fig. 4. On bisection of the kidney, no gross pathologic process was visible. The pelvis was normal. A probe is shown passed from the kidney down the normal ureter into the ileal bladder.

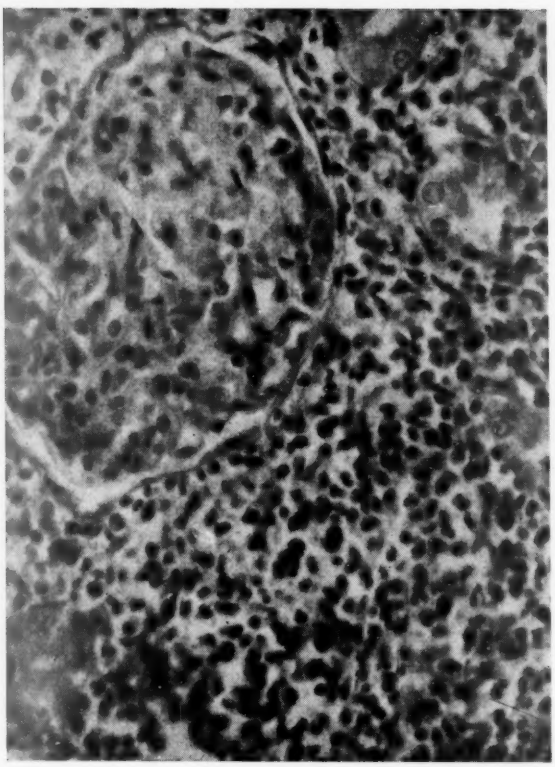

Fig. 5. Membranous glomerulitis. The capillaries have thickened walls. The surrounding stroma contains chronic inflammatory infiltrate. (×400; reduced ⅔.)

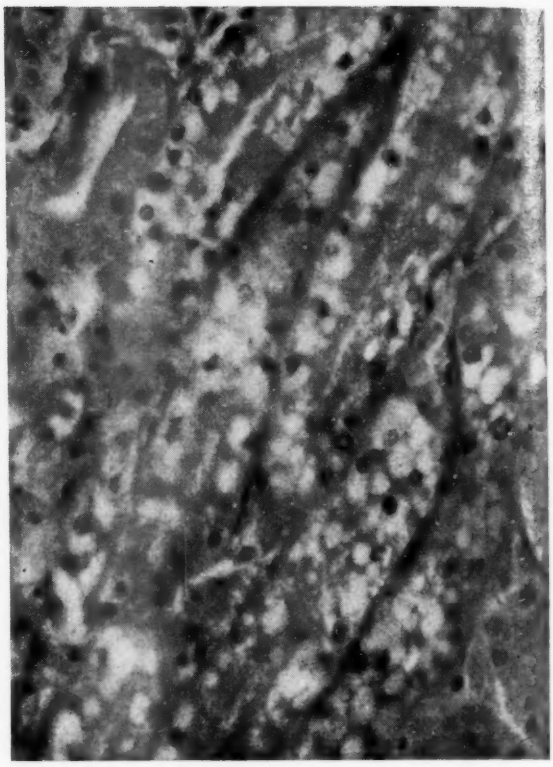

Fig. 6. Renal tubular changes. There is vacuolation of the tubular epithelium and protein material within the tubular lumina. (×400; reduced ⅔.)

serted into the sigmoid. The right and left ureters were transplanted into the ileal loop at monthly intervals.

The first dog (No. 12) died in 7 days. The changes in the electrolyte levels from the time of the second transplantation were blood urea nitrogen (BUN), 18 to 169 mg. per cent; serum chlorides, 110 to 109 mEq. per liter; serum sodium, 142 to 145 mEq. per liter; serum potassium, 3.6 to 3.1 mEq. per liter; and carbon dioxide combining power, 21 to 19 mEq. per liter.

The second animal lived 28 days. The electrolyte changes were BUN, 20 to 330 mg. per cent; serum chlorides, 127 to 83 mEq. per liter; serum sodium, 156 to 119 mEq. per liter; and carbon dioxide combining power, 21 to 6 mEq. per liter.

The pathologic reports on the 2 dogs were quite different. In Dog 12 a stricture of the right ureter was found, 1 inch long, close to the ileal loop. The kidney was one-half the size of the opposite kidney. Pathologic section showed protein tubular casts in the kidney and a chronic periureteritis partially obliterating the lower end of the ureter. Dog 14 had an acute bilateral necrotizing papillitis, acute pyelonephritis, and acute ureteritis (Figs. 7 and 8).

Experiment 4. Our fourth experiment was a two-stage procedure. An ileal loop was isolated as in the previous experiment. One month or more later, both ureters were transplanted into the ileal loop. In 7 animals the postoperative course was similar. The blood urea nitrogen rose gradually, but not as fast or as high as when the ureters were transplanted at different stages. The serum chloride and serum sodium levels remained normal. Serum potassium levels fell to a low normal. Carbon dioxide combining power fell in all cases and the average was 13.8 mEq. per liter. The average life of these animals was 23 days postoperatively.

The eighth dog followed a different pattern. There was an interval of 7 weeks be-

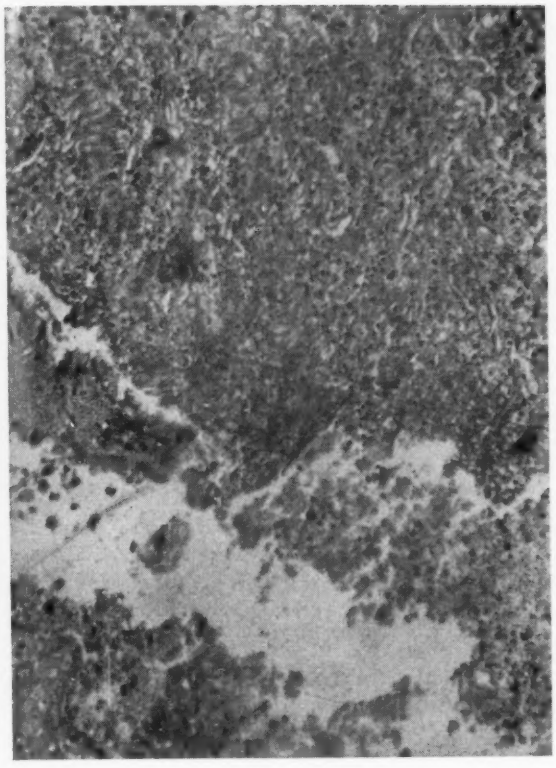

Fig. 7. Necrotizing papillitis. Note the ulceration of the renal papillae and the acute inflammatory reaction present. (×150; reduced ⅔.)

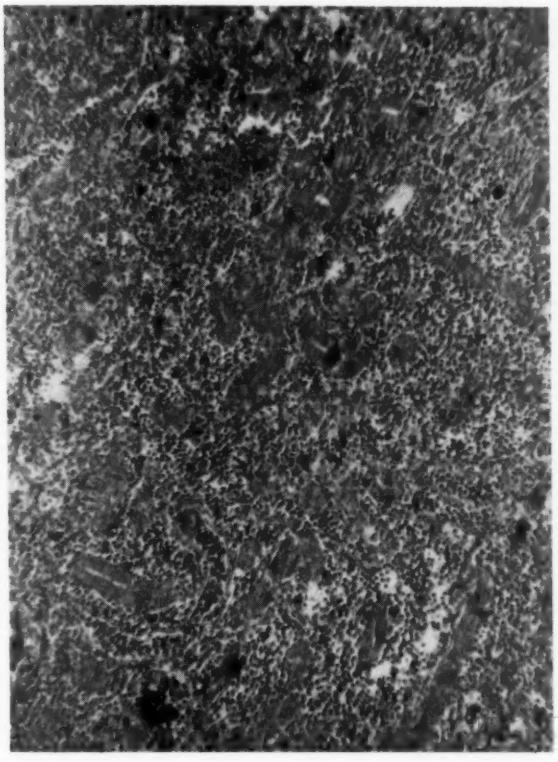

Fig. 8. Acute pyelonephritis. The interstitial tissues are diffusely infiltrated by acute inflammatory cells. (×150; reduced ⅔.)

tween the ureteral transplants. The BUN at the first stage was 11 mg. per cent, and 20 mg. per cent at the second stage. Weekly BUN determinations were taken for 6 weeks, and the results were 26, 50, 52, 42, 36, and 32 mg. per cent. The carbon dioxide combining power fell as the BUN rose, and rose as it fell. The animal began gaining weight, looked better, and ate better. We discontinued our electrolyte studies temporarily, and the animal died suddenly, 2½ months after the ureteral transplant. Postmortem examination showed an incomplete stricture at the lower end of the right ureter. Histologic section of the right kidney showed an acute pyelitis and ureteritis. On the left side there was a huge hydroureter and a chronic pyelitis and ureteritis.

Experiment 5. On Nov. 17, 1959, we formed an ileal loop attached to the sigmoid in animal (No. 21). Bilateral ureteral transplants of both ureters were carried out on December 22. The animal weighed 22 pounds. December 28, the BUN was 50 mg. per cent. Repeat electrolyte studies were performed on January 18 and were BUN, 76 mg. per cent; serum potassium, 3.9 mEq. per liter; serum sodium, 145 mEq. per liter; serum chlorides 120 mEq. per liter; and carbon dioxide combining power, 13 mEq. per liter, the animal weighed 18 pounds and was in poor condition. On January 19, we again operated on this dog, disconnected the ileal loop from the sigmoid, and performed an end-to-side anastomosis of the ileal loop to the bladder. On March 30, 1960, the animal had regained its normal weight. The BUN had fallen to 28 mg. per cent. The animal looked well. On June 25, the BUN was 22 mg. per cent and the animal weighed 23 pounds.

SUMMARY

This is a preliminary report. We are aware that our series is not large enough to allow us to draw definite conclusions. We do be-

lieve that some pertinent observations are in order.

1. A loop of ileum acting as a urinary conduit between the ureter or kidney and bladder is compatible with a normal existence in animals and human beings. The electrolyte balance is not disturbed.

2. A loop of ileum used as a urinary conduit between the ureters and sigmoid has 3 disadvantages, (1) the reabsorptive area is greatly increased (ileal loop and sigmoid), (2) the urinary tract is exposed to an ascending infection from the sigmoid, and (3) the pressure in the urinary tract is increased because the pressure in the sigmoid is greater than that normally found in the urinary tract.

3. The active peristalsis in the isolated ileal loop does not prevent ascending infection.

4. A valve at the distal end of the ileum does not prevent ascending infection but appears to prevent regurgitation of fecal content into the ileal loop.

5. A valve at the distal end of the ileum appears to at least temporarily add an obstructive element to the free flow of urine from the ileal loop into the sigmoid.

6. Transplantation of the ileal loop into the sigmoid is followed by infection of various degrees in the ureter, kidney pelvis, and kidney. This tends to electrolyte imbalance and a rapid and great rise in the BUN, and terminated in death in all our animals.

7. The process can be reversed by disconnection of the ileal loop from the sigmoid and re-insertion of it into the urinary bladder. The animal regains its health and electrolyte balance is re-established.

8. It appears that diverting urine by way of an ileal conduit to the sigmoid is not a satisfactory procedure.

9. Formation of the ileal bladder as a first stage and allowing time for all edema to subside appeared to increase the life span of our experimental animals.

The biochemical, bacteriologic, and pathologic studies were graciously carried out gratis by the Departments of Biochemistry, Bacteriology, and Pathology at the St. Boniface Hospital. All photographic work was done by Mr. Stanford, in the Photographic Department at the St. Boniface Hospital. Miss Nancy Joy, medical artist from the School of Medicine, made the diagram for our case of calycoileoneocystostomy. Johnson & Johnson donated suture material to help us with our project. We are grateful to a number of anaesthetists, particularly Dr. Joseph Hayakawa, who donated their services for our operations.

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The percutaneous transhepatic approach of the portal vein in man in the determination of patency of splenorenal shunts

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A great advance was achieved in the treatment of portal hypertension with the introduction of portacaval shunts. With the aid of splenoportography, we can easily determine the patency of a portacaval shunt, but for the splenorenal shunt we have to rely upon operative portography. This is one argument voiced by those who are opposed to the use of splenorenal shunts. We cannot demonstrate the patency of the splenorenal shunt with the same ease as we demonstrate that of the portacaval shunt. Bierman and his group[2] in 1952 presented the percutaneous transhepatic puncture of the portal vein and in 1955[1] performed catheterization of the portal vein, but they concluded that catheterization was inadequate for portography because of the small caliber of the catheter that had to be used. Since 1958,[3] we have been performing the percutaneous transhepatic puncture of the portal and hepatic veins in all cases of portal hypertension in order to determine the portal and hepatic pressures and draw blood for biochemical studies. The rationale of the percutaneous transhepatic approach of the portal or hepatic veins is that the portal vein branches after entering the liver, with one branch for the right lobe and the other for the left. In a transverse cross section which passes through the xiphoid process, the largest branches of the portal vein are seen to lie to the right of the midline in an angle which varies from 10 to 45 degrees (Fig. 1). At this point the liver is in direct contact with the anterior abdominal wall, so that no organ is interposed between. If at this point a long needle is inserted through the skin at an angle of 20 degrees to the right, one of the branches of the portal vein will probably be entered. If the needle is inserted 5 to 10 degrees cranially and 10 to 15 degrees to the right, one of the hepatic veins may be entered. In 17 patients with splenorenal shunts a percutaneous transhepatic approach of the portal vein was tried and portography was performed either directly through the needle or after catheterization of the portal vein.

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TECHNIQUE

The patient, in a fasting condition, was well sedated with a mixture of 100 mg. of Demerol, 25 mg. of chlorpromazine, and 50 mg. of promethazine given intramuscularly 45 minutes before the procedure. The patient was then placed in a supine position on the x-ray table with the hands under the head and the skin was prepared with an antiseptic solution. An infiltration of all the planes of the abdominal wall was carried out with a solution of 1 per cent procaine at the site of puncture which was just to the right of the xiphoid process. The needle, which has to be long and proper for catheterization, was made by one of us. It was

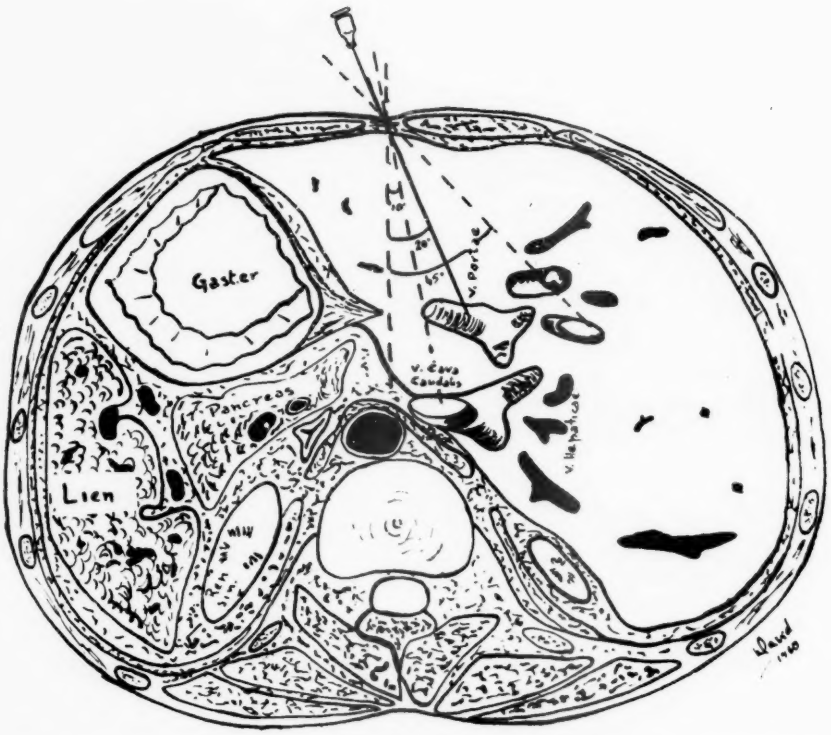

Fig. 1. A transverse cross section through the xiphoid process which shows the positions of the large blood vessels in the liver.

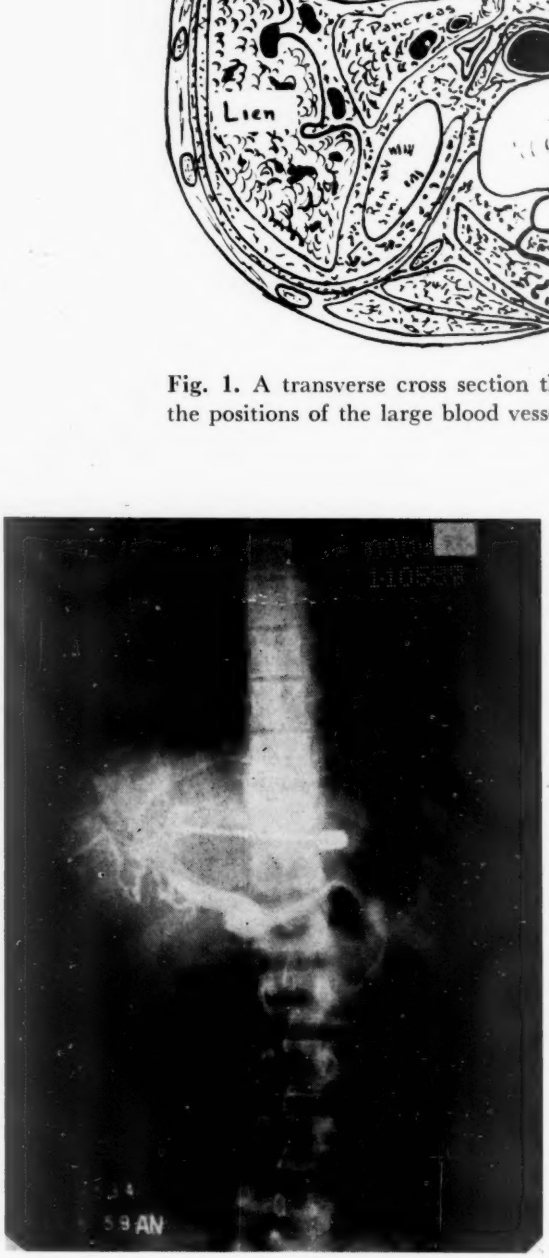

Fig. 2. The first film, taken immediately after injection of contrast medium (20 ml.) through polyethylene tube.

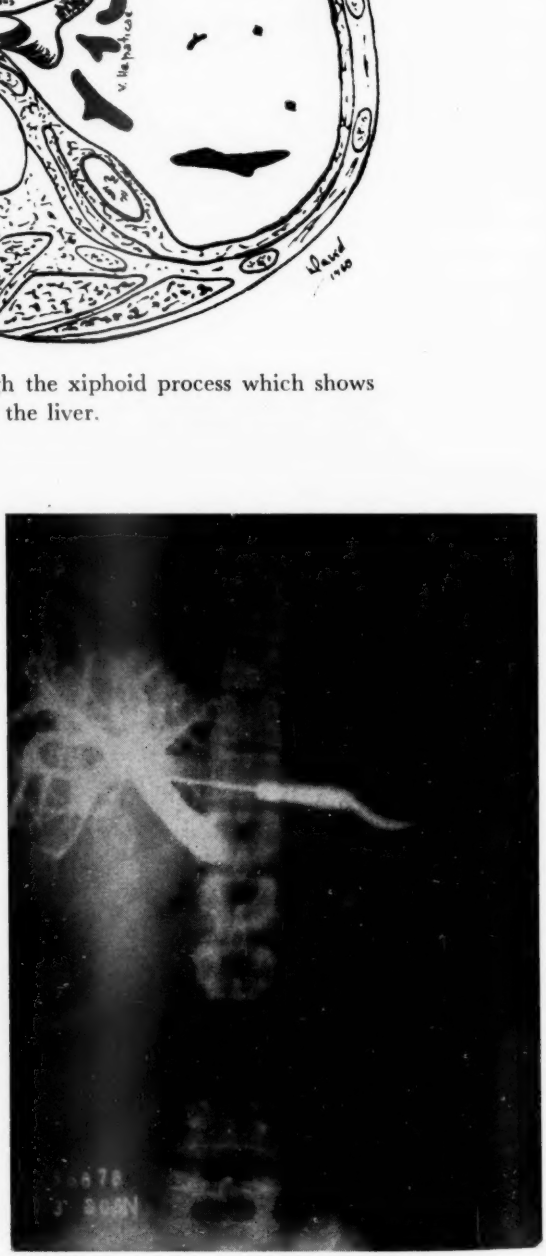

Fig. 3. The film taken after the contrast medium had been injected directly through the needle. There has been no backflow to splenic vein.

18 cm. long, 1.8 mm. in diameter, and had a well-curved Huber point. Thus, a catheter threaded through the needle would come out through the point which was already angled to permit progression of the catheter into the vein. Without this posterior curving of the needle point it would not be possible to thread a catheter of sufficient width through the needle into the vein. A No. 60 polyethylene catheter was used. The needle was inserted completely into the liver mass at an angle of 20 degrees to the right. A syringe was adapted to the needle and the needle then slowly pulled back while the syringe was being aspirated. Blood flowing into the syringe indicated that the needle had penetrated a vein, probably one of the branches of the portal vein. When blood flowed freely in and out of the syringe, it meant that the opening of the needle was in a good position in the vein. Before threading the catheter we had to be sure that the vein punctured was the portal vein. This was very easy to confirm in patients with portal hypertension, even in those who had a splenorenal shunt, since the portal pressure in these cases is much higher than the hepatic pressure. We found the hepatic pressure to vary from 60 to 120 mm. H_2O and was rarely higher than 150 mm. H_2O, considering the base line at the posterior axillary line. However, if we were still in doubt venography was performed by injection of a small amount of contrast medium (5 to 10 ml.). The polyethylene tube was then threaded through the needle and easily advanced, down the portal vein, generally going to the splenic vein. Twenty milliliters of contrast medium, such as Opacoron 400, was then injected rapidly through the polyethylene tube with a 20 ml. syringe and the first film was taken just at the end of the injection (Fig. 2). The catheter was then removed and a syringe with an intermediate rubber tube adapted to the needle. Thirty milliliters of the contrast medium was rapidly injected for the second film. The reason that 2 films should be taken is that if the contrast medium is injected directly into the needle, it will not always flow back against

Fig. 4. In this instance the contrast medium has been injected through the polyethylene catheter.

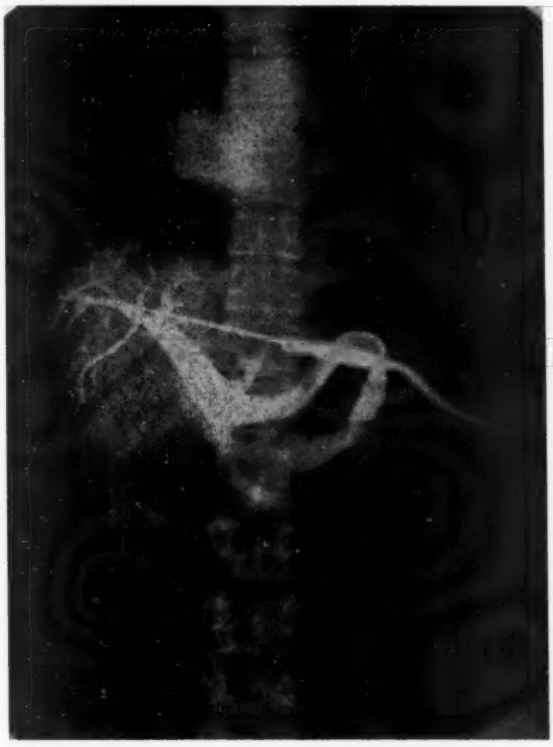

Fig. 5. Same patient as in Fig. 4. Contrast medium was injected directly into needle. Portal vein, splenic vein, left renal vein, inferior vena cava, and right atrium of the heart can be seen.

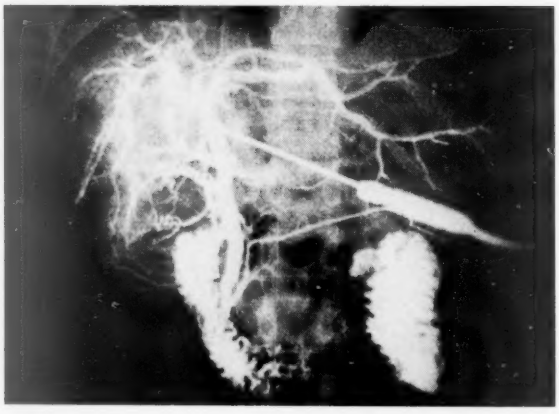

Fig. 6. Contrast medium inadvertently injected into biliary tract.

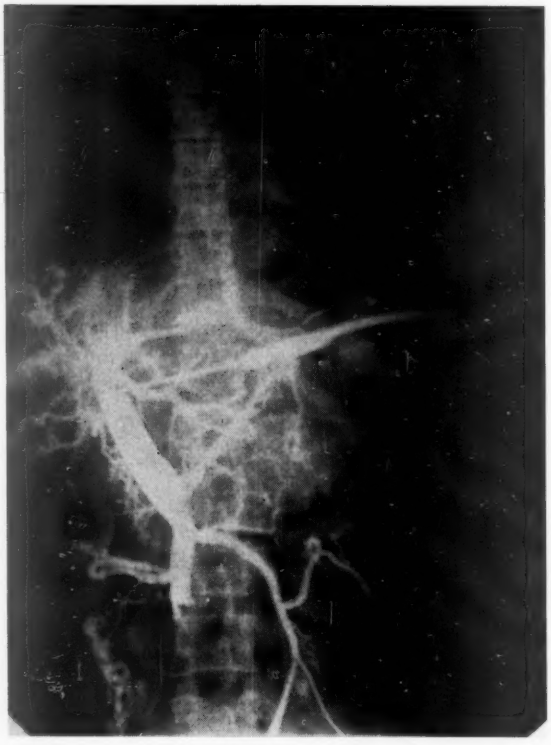

Fig. 7. Case in which shunt was thrombosed.

the blood stream in the portal vein to the splenic vein (Fig. 3). If the contrast medium goes by this way through the portal vein to the splenic vein the picture will be much clearer than the one obtained by injection of contrast medium through the polyethylene tube because the relatively small size of the tube will not permit a rapid introduction of the contrast medium (Figs. 4 and 5).

If the portal vein cannot be punctured with the angulation recommended, other angulations can be tried. There will be no harm in performing several punctures while trying to locate the right vein.

RESULTS

We attempted to demonstrate the splenorenal shunt roentgenographically in 17 patients. In 2 cases we failed to puncture the portal vein. Of the 15 cases in which the portal vein was punctured, catheterization was not attempted in 1 and in 6 cases our catheterization efforts failed, probably because of the small caliber of the vein that was punctured. In these 6 cases venography was performed directly through the needle. In one there was no backflow of the contrast medium along the portal vein to the splenic vein and in the other, the biliary tract was incidentally shown (Fig. 6). In the patient in whom catheterization was not attempted, the introduction of the contrast medium directly through the needle showed a portal vein with many collaterals, such as the left gastric vein, with a venous plexus going to the esophagus, and the superior and inferior mesenteric veins denoting thrombosis of the shunt (Fig. 7). Of the 8 patients catheterized, in 4 the catheter went into the splenic vein and in all of them radiography showed the shunt to be patent. In 1 case the roentgenogram showed the catheter to have entered the biliary tract, and in the remaining 3 cases the shunt was thrombosed. Therefore, of the 17 cases presented, the splenorenal shunt was shown to be patent in 4, it was thrombosed in 6, and in the remaining 7 cases either the portal vein could not be punctured, or it was punctured but could not be catheterized, or the biliary tract was punctured inadvertently.

DISCUSSION

This method is relatively simple and safe for the patient. There is no serious risk of internal bleeding either due to rupture of the liver substance or bleeding from the tract of the needle in the liver. Bierman[1] refers to 144 punctures without a single accident. We performed 86 punctures with a large

needle and no serious bleeding occurred. We have performed punctures of the portal and hepatic veins in the same patient and sometimes have had to make several attempts before the desired vein was punctured. In the few cases in which the patient underwent operation on the day after the puncture, a certain amount of blood was found in the abdominal cavity varying roughly from 50 to 150 ml., but because splenoportography had been performed at the same time in these patients, we could not determine exactly how much of the blood was from the liver and how much was from the spleen. Four patients of the 86 mentioned had small hematemesis after the punctures when they returned to the ward. We attributed this small amount of bleeding to a traumatic hemobiliary fistula caused by the needle. None of them needed blood transfusion, had fall of blood pressure, or had a rise of the pulse rate. None of them experienced bleeding after repeat puncture later in the postoperative period. None of the 17 patients discussed in this paper had any accident. The patients should breathe smoothly and if they have nausea or vomiting the needle should promptly be removed to prevent any possible rupture of the liver substance.

There is no danger of bile peritonitis as long as there is no obstruction to the free flow of bile.

With the patients well sedated as we recommend the puncture is well tolerated and most of them remain practically asleep.

SUMMARY

We wish to call attention to the fact that one of the arguments against the splenorenal shunt is the fact that one cannot demonstrate the patency of the shunt unless a laparotomy is performed. Using the percutaneous transhepatic puncture of the portal vein complemented with catheterization, we were able to demonstrate the splenorenal shunt by means of roentgenograms. In 17 cases, the patency of the shunt could be demonstrated in 4, the shunt was thrombosed in 6, and in the remaining 7 either the portal vein could not be punctured or catheterized or the biliary tract was inadvertently punctured. The method is a simple and safe one.

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The relation of blood pH to the movement of ammonia into cerebral and peripheral tissues during hypothermia

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A mechanism for the role of ammonia in hepatic coma has been proposed on the basis of the fact that ammonia is taken up by the peripheral and cerebral tissues as the level of ammonia rises in the blood.[3, 6] The ammonia that enters the brain tissue is considered to react with the alpha-ketoglutarate of the Krebs's cycle to form glutamate. This process depletes the Krebs's cycle of intermediates, which results in the inability of the brain to oxidize substrate and generate energy, and this in turn causes coma. A considerable amount of evidence has now been adduced to support this theory.[1, 2, 4, 7, 8] This mechanism offers an explanation of the toxic intracellular action of ammonia. It was assumed that diffusion over a gradient from blood to tissue was the primary factor in the movement of ammonia into the cells. Another mechanism which stimulates the uptake of ammonia by tissues has been proposed.[17, 23] This is based on the well-known fact that the ammonium ion cannot penetrate tissues, but that unionized ammonia can pass through the cell membrane.[13] It is also known that the proportion of unionized ammonia is increased with alkalinity, and is decreased under acid conditions. It has therefore been suggested that the hyperventilation associated with hepatic coma causes an alkalosis which stimulates the movement of ammonia from the blood into the tissues, thus aggravating the coma.[12] An opportunity to test this attractive hypothesis was offered by a series of observations which we have been making on the events which transpire during refrigeration anesthesia.[10] Over periods of up to 7 hours we have measured the pH and ammonia content of arterial, peripheral venous, and jugular blood, and can therefore correlate arterial-venous differences with the pH of the blood at the moment. Long-term fluctuations of blood pH up to 0.50 U. have been observed, and the concomitant changes in ammonia have been recorded.

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METHODS

A catheter was threaded through the femoral vein to the bifurcation of the vena cava, well below the renal outflow, to obtain venous blood and arterial blood was sampled through a catheter in the common carotid artery. The jugular bulb blood was sampled through a catheter threaded through the superior vena cava after the chest was opened. Ammonia was measured by a modification of Seligson's procedure[19] in duplicate. The maximum difference from the mean of any pair of observations reported was 0.06 gamma per milliliter. The pH was measured anaerobically at 37° C. by means of a Leeds-Northrop pH meter with a special blood electrode. The patients were put into a hypothermic state by immersion in ice water, about 30 minutes after the

first observation. Rectal temperatures of 28° to 32° C. were maintained. Eleven patients were studied. Only data on simultaneously drawn samples of blood are compared, and no values are included which represent the first 2 minutes after release of occlusion of cerebral vessels in view of marked and transient pH variation seen during this period.[5]

RESULTS

Fig. 1 shows the relation between arterial level of ammonia, arteriovenous difference of ammonia, and pH of the arterial blood. The pH of arterial blood is recorded because this has been proposed as the critical factor in the movement of ammonia into the tissues.[20] The arteriovenous difference of the extremity, in this case the lower limb, varied between a positive value of 0.63 gamma per milliliter and a negative value of approximately 0.15 gamma per milliliter. During the same period the pH of the blood varied between 7.30 and 7.50. The data have been so plotted that if the curves parallel each other, they indicate a positive correlation. It can be seen from Fig. 1 that there is no significant correlation between the fluctuations in peripheral arteriovenous difference of ammonia and the variations in arterial pH. On the other hand, there is a clear correlation between the arterial level and the peripheral arteriovenous difference of ammonia. Fig. 2 is another set of observations which shows a lack of correlation between arterial pH and arteriovenous difference at the same time that a remarkably good correlation can be seen between arterial level and arteriovenous difference. It is also of interest that alteration in the ammonia economy of the extremity covered the range between a positive difference of 0.3 gamma per milliliter to a negative difference of 0.7 gamma per milliliter, so that, as in the first case, a shift in direction of movement of ammonia into the tissues occurred during a time when the arterial pH fluctuated only minimally. The arterial ammonia level which correlated with these shifts varied over a range of approximately 50 per cent.

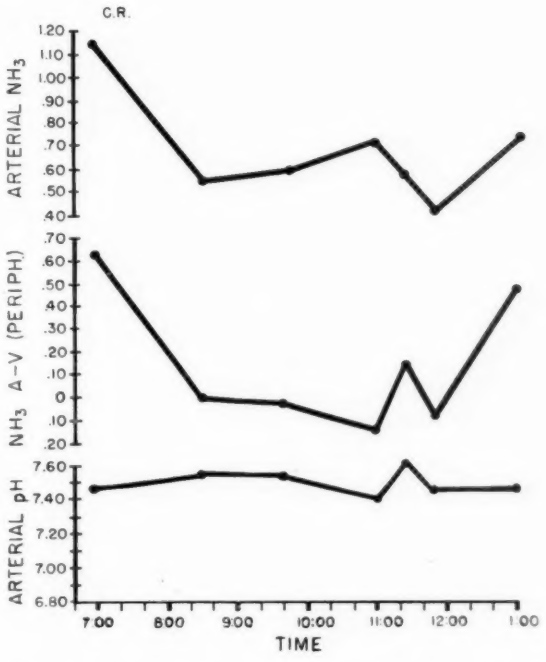

Fig. 1. Arterial pH, ammonium concentration, and arteriovenous difference of ammonium across the lower extremity in Patient C. R.

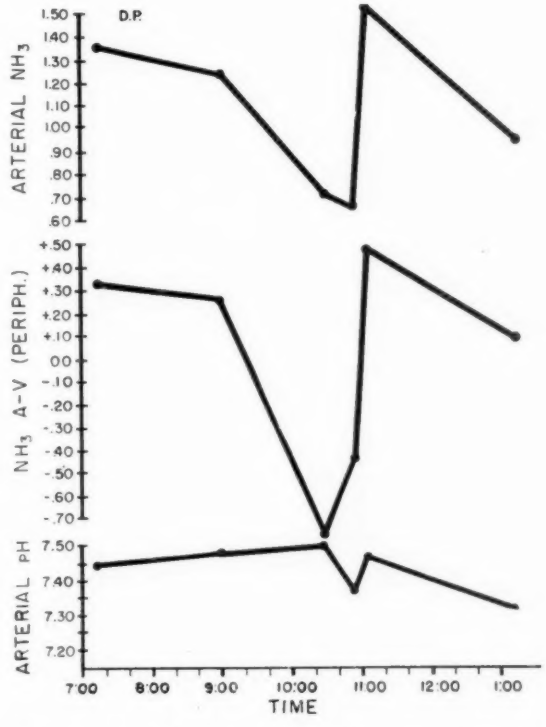

Fig. 2. Arterial pH, ammonium concentration, and arteriovenous difference of ammonium across the lower extremity in Patient D. P.

Table I. *Protocol for Patient J. P., 7-year-old boy, on Oct. 11, 1957*

Time	*Source*	*pH*	*NH_4*	*Temperature (C.)*
7:52 A.M.	Femoral artery	–	2.18	37.6°
7:59	Inferior vena cava	7.36	2.17	
	(hypothermia)			
11:06	Femoral artery	7.64	0.66	28.0°
	Inferior vena cava	7.52	0.59	
	Jugular vein	7.32	1.06	
11:20	Clamp on inferior vena cava			
	Clamp on superior vena cava			
11:22	Femoral artery	7.66	0.80	28.5°
	Inferior vena cava	7.54	1.21	
	Jugular vein	–	1.24	
11:28	Removal of both clamps			
	Jugular vein	7.54	0.91	
11:32	Femoral artery	7.48	1.00	28.5°
	Inferior vena cava	7.52	0.95	
	Jugular vein	7.58	0.95	
11:38	Femoral artery	7.50	1.09	28.5°
	Inferior vena cava	7.38	0.62	
	Jugular vein	7.48	1.03	
11:44	Femoral artery	7.61	0.74	28.5°
	Inferior vena cava	7.54	0.76	
	Jugular vein	7.50	0.86	
11:49	Femoral artery	7.54	0.79	29.5°
	Inferior vena cava	7.55	0.77	
	Jugular vein	7.50	0.54	
	(return to normal temperature)			
5:00 P.M.	Femoral artery	–	0.83	37.0°
	Inferior vena cava	–	0.77	

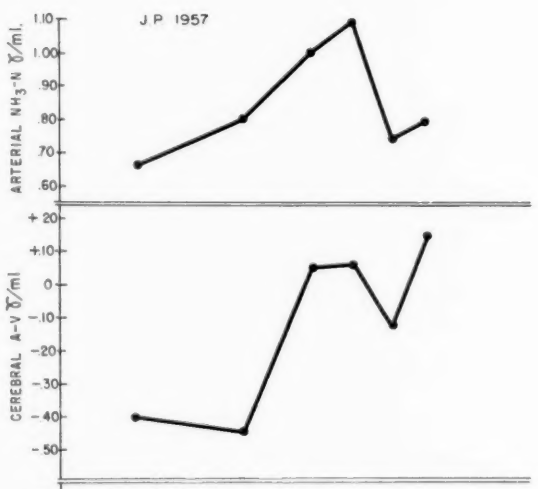

Fig. 3. Arterial pH, ammonium concentration, and arterial-jugular difference in Patient J. P.

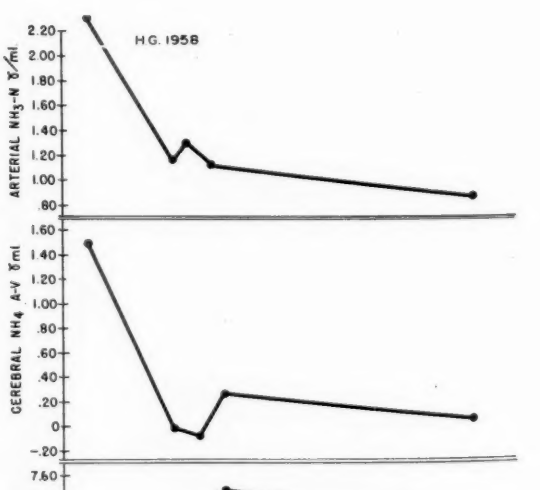

Fig. 4. Arterial pH, ammonium concentration, and arterial-jugular difference in Patient H. G.

Fig. 3 shows observations on the cerebral arteriovenous difference of ammonia in relation to arterial level and jugular pH. Again there is a good correlation between arterial level and arteriovenous difference. In this case there is somewhat better correlation between arterial pH and cerebral uptake than there was between peripheral uptake of ammonia and pH in Figs. 1 and 2. On the other hand, the general trend (increase or decrease) of arteriovenous difference of ammonia parallels the course of variation of arterial level better than it fits the variation of arterial pH. The greatest change in pH is associated with the least alteration in arteriovenous difference. The protocol which was used for this typical experiment is given in Table I.

Fig. 4 shows the cerebral arteriovenous differences of ammonia in another patient and a clear inverse relation can be seen between pH and variation of arteriovenous difference across the brain. This covers a sufficient number of points to make the relation more than fortuitous. The fluctuations in arteriovenous difference across the brain are closely related to the arterial ammonia changes. Every change in uptake vector is in the same direction as arterial vector change.

Fig. 5 is a scattergram of arterial pH values versus arteriovenous difference taken from 11 cases. There are 62 values for peripheral and 34 values for cerebral arteriovenous difference. No positive correlation can be seen between the arteriovenous difference, either cerebral or peripheral, and the pH of the blood. Table II gives the correlation coefficients for Fig. 5, and for Figs. 6 and 7 which show the relation between arterial ammonia level and peripheral and cerebral arteriovenous difference, respectively. A negligible negative correlation exists between arterial pH and peripheral arteriovenous difference of ammonia, but the negative correlation between cerebral pH and cerebral arteriovenous difference may be significant. The positive correlations between arterial level and cerebral and peripheral arteriovenous difference of ammonia are significant at the 0.0001 and 0.02 probability levels.

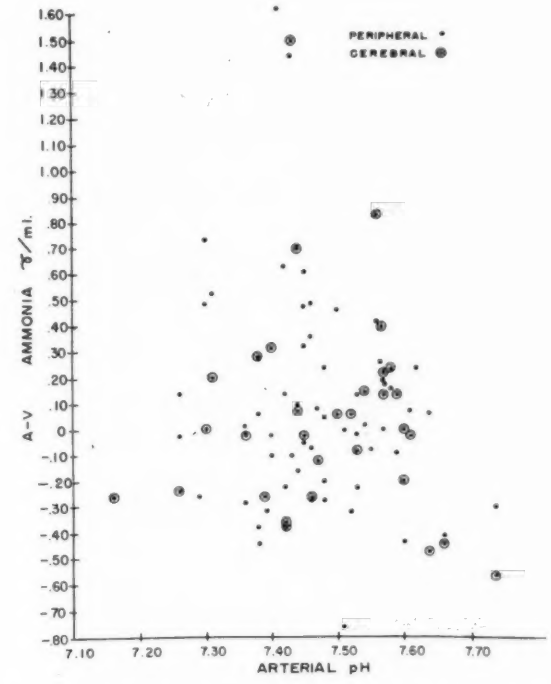

Fig. 5. Lack of relation between arterial pH and cerebral or peripheral arteriovenous difference of ammonium.

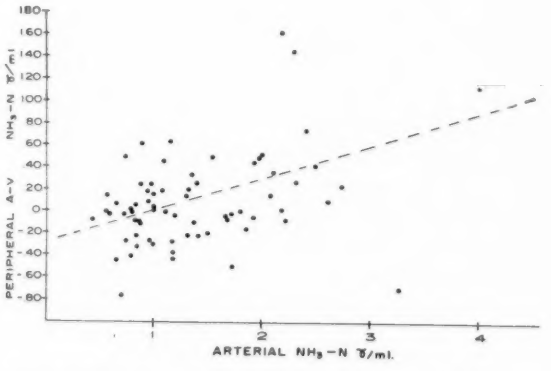

Fig. 6. Peripheral (lower extremity) arteriovenous difference of ammonium as a function of arterial level of ammonium.

It should be noted at this point that plots of venous pH data showed the same trends as the arterial data given. Fig. 8 shows the lack of correlation between venous pH and arteriovenous difference.

DISCUSSION

These data, and more fragmentary observations on a number of other patients,

Table II. *Correlation coefficients for cerebral and peripheral uptake of ammonia*

	n	*r*	*p*
pH (peripheral uptake)	62	−0.19	> 0.10
pH (cerebral uptake)	34	−0.50	< 0.005
Arterial ammonia (peripheral uptake)	62	+0.31	< 0.02
Arterial ammonia (cerebral uptake)	34	+0.61	< 0.0001

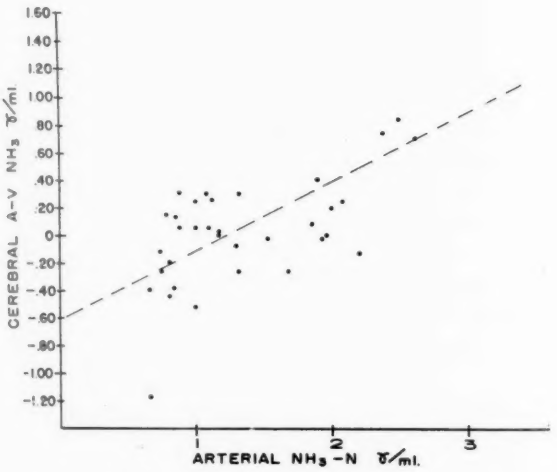

Fig. 7. The cerebral arteriovenous difference of ammonium as a function of the arterial ammonium level.

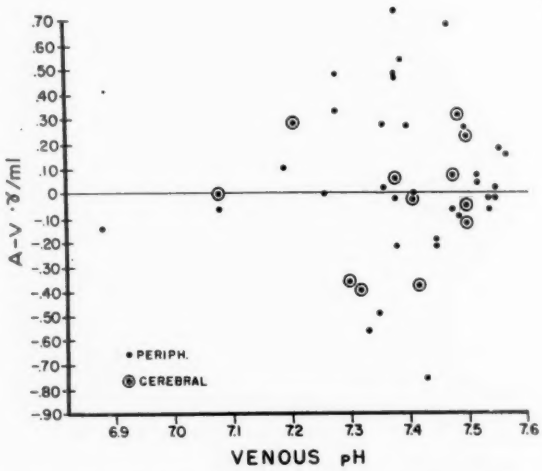

Fig. 8. Lack of correlation between venous pH and peripheral and cerebral arteriovenous difference of ammonium.

suggest that there is little effect of the pH of the blood upon the arteriovenous difference of ammonia across tissues. The experiments of Jacobs and Parpart[13] showed indirectly (as indicated by osmotic swelling) an increase in red cell ammonia in vitro as the medium was made more alkaline, but no direct measurements of this phenomenon have been reported. The work of Jacquez and others[11, 18] on the vapor pressure of ammonia in vivo leaves no doubt as to the effect of pH in altering the proportion of ammonia in the gaseous or diffusible form. Our own experiments[21] with distribution of ammonia between red blood cells and plasma suggest a slight effect of pH, but cannot account for the fall in blood ammonia seen clinically on alkalization and the rise seen in acidosis. Lawrence and associates[16] demonstrated alterations in the blood ammonia of dogs infused with acid or alkali or subjected to hyperventilation. When the pH of the blood was made strongly acid, the blood ammonia level was considerably higher than when the pH of the blood was alkaline. This was cited as evidence that the ammonia had moved into or out of the tissues under the circumstances of the experiment. Examination of the data, however, shows the interesting fact that the peripheral arteriovenous difference at acid pH was the same for comparable total ammonia levels as the difference at an alkaline pH. In other words, the only data which might indicate actual movement of ammonia into the tissues in the experiments of these authors were not influenced by the pH. The arterial level of ammonia was inversely related to the pH, and this is a different matter. It suggests that a general mechanism of removal of ammonia from the arterial blood may be affected by the pH of the blood; that is, the removal of ammonia by hepatic formation of urea was probably impaired by the acidity of the blood. This is illuminated by experiments reported from Krebs's laboratory.[15]

It was shown, in measuring the formation of urea from ammonia by liver slices in vitro, that both the pH of the medium and the bicarbonate content had a remarkable in-

fluence upon the rate of urea synthesis; that alkaline conditions favored synthesis of urea and acid conditions inhibited it. These effects were manifest within the physiologic range of pH. The recent demonstration by Jones and Lipmann[14] that carbamyl phosphate synthesis, the first step in the incorporation of ammonia into urea, is at a maximum at approximately pH 8 may identify the pH-sensitive enzymatic step which is implied in Krebs's experiments. The effect, therefore, of pH upon the level of blood ammonia demonstrated by Lawrence and colleagues[16] is not an effect of pH on the movement of ammonia into the tissues, but is more likely an effect of pH upon the synthesis of urea by the liver. The negative correlation between pH and arteriovenous difference which can be observed is consistent with this interpretation. When the pH is acidic, urea synthesis slows down, the blood ammonia level rises, and tissues take up ammonia. When the pH rises, urea synthesis improves, the arterial ammonia level falls, and less ammonia is taken up by tissues. The conversion of even the equivalent of 5 gamma of ammonia per milliliter of blood, a formidable amount, to urea would raise the blood urea nitrogen by 0.5 mg. per 100 ml., a negligible, and probably undetectable, difference. The only reference to blood urea nitrogen changes during pH manipulations is by Stabenau and his group[20] who say that there was "no striking difference in rate of BUN elevation," but give no data. The therapeutic benefits reported from the use of acidifying measures probably can be attributed to the fact that alkalosis in general is deleterious to neural function and tends to aggravate neurological signs.

We appreciate the fact that alterations in acidity of the blood might affect ammoniagenic coma, as suggested by the Sloan-Kettering group. We take issue merely with the interpretation of the results, and believe that a more physiologic interpretation can be derived than that the effect of acidification is upon the movement of ammonia into tissues. In reality the movement of ammonia into tissues is apparently governed

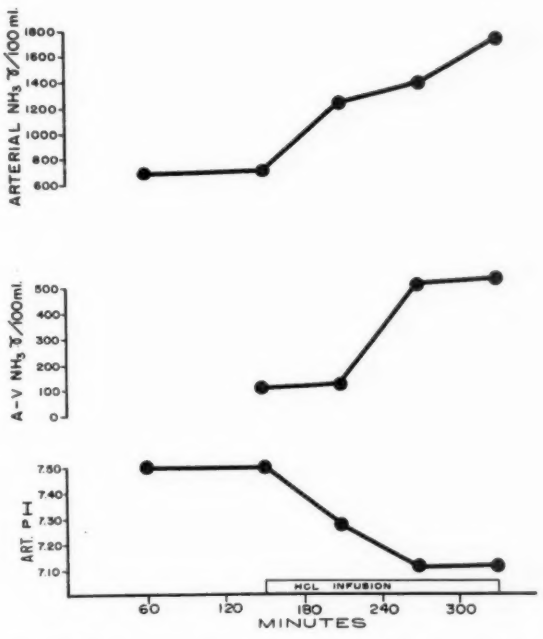

Fig. 9. Relation of peripheral arteriovenous difference of ammonium to arterial concentration and pH of arterial blood plotted from Table IV of Stabenau and associates[20] (Dog 1).

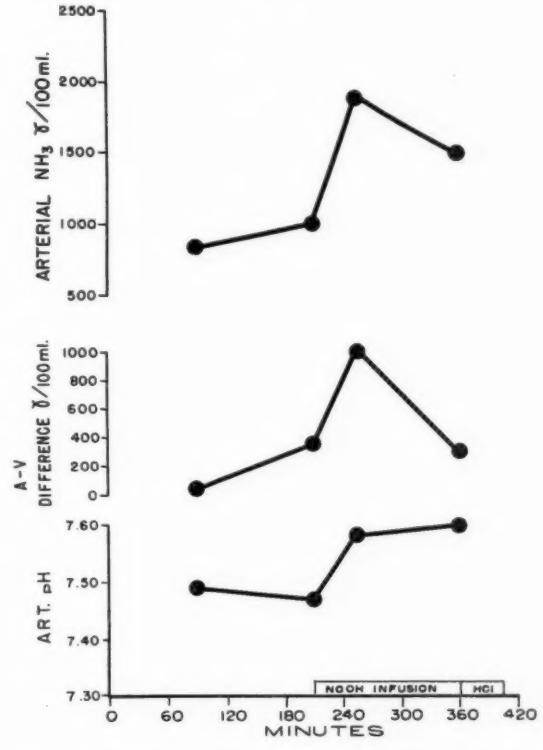

Fig. 10. Relation of peripheral arteriovenous difference of ammonium to arterial concentration and pH of arterial blood plotted from Table IV of Stabenau and associates[20] (Dog 3).

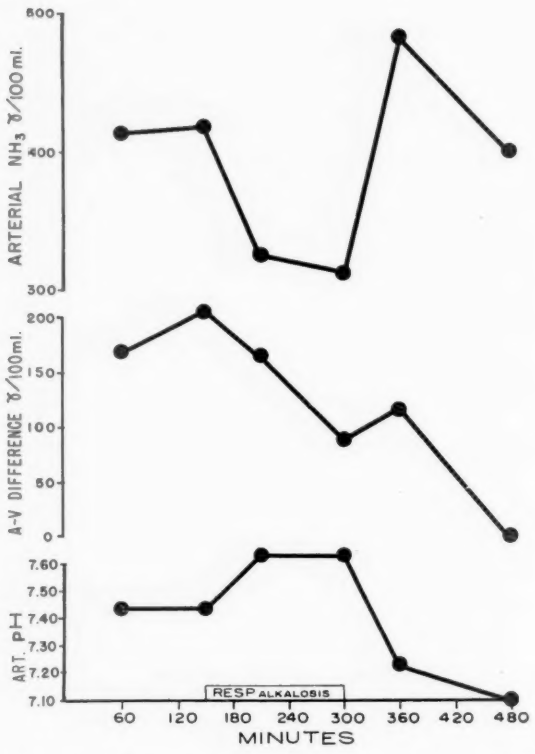

Fig. 11. Relation of peripheral arteriovenous difference of ammonium to arterial concentration and pH of arterial blood plotted from Table IV of Stabenau and associates[20] (Dog 4).

mainly by the arterial level of ammonia. This is not to say that there is a particular level of ammonia which stimulates a particular rate of movement into the tissues. This again is conditioned by many factors, including, perhaps significantly, the actual level of ammonia within the tissues at the time. In the same patient, however, followed over a long period of time, the movement of ammonia into the tissues is related to the arterial level of ammonia, not to the pH of the blood. The experiments[20, 24, 25] in which overwhelming amounts of ammonium salts were administered to animals and wide deviations of pH were instituted do not appear to be germane to the problem of the role of pH under physiologic or even pathologic conditions. The fact that enormous levels of ammonia appeared in the brain tissue at high blood pH is expected from the experiments of Jacobs. Similar results can be obtained by performance of diffusion experiments across semipermeable membranes.

The problem at hand is not whether or not ammonium salts ionize. The problem is whether the movement of ammonia into tissues is determined mainly by arterial pH or by arterial level of ammonia. Obviously it would be preferable to have measurements of blood flow to make a quantitative decision. Nevertheless, there appear to be sufficient data at hand to make a fair conclusion possible.

Since there is material in the literature which purports to show a significant effect of pH upon movement of ammonia into tissues, it is necessary to determine whether the proffered data actually support this hypothesis. Stabenau and colleagues[20] report a relation between tissue distribution and blood pH, and the figures presented seem to bear this out. Closer scrutiny of Fig. 2 in their report shows that the data of their Table IV have not been included. When they are included, the relation between pH of blood and muscle-blood distribution becomes considerably less significant. The same question can be raised for their Fig. 3 which loses its significance in demonstrating an effect of pH upon brain-blood distribution when the data of their Table IV are added to it. Table III shows that muscle content of ammonia is the same in respiratory acidosis as in metabolic alkalosis; even in respiratory alkalosis there is no significant difference from the controls in muscle content of ammonia.

The information on tissue and blood concentration is presented mainly as ratios. The only absolute values, Table VI, show a number of discrepancies between data and discussion which are worth considering. The sodium hydroxide infusion produces a higher blood ammonia level than hydrochloric acid infusion (against the hypothesis) at the same time as an apparent change in the "right" (according to hypothesis) direction occurs for brain and muscle concentration. Furthermore, respiratory alkalosis results in a blood level which falls even as the tissue levels also fall (but they should rise if the ammonia which left the blood entered the tissues).

It is instructive to plot the arteriovenous differences which can be calculated from the data presented in this paper (Table IV) as we have plotted ours. Figs. 9, 10, and 11 have been so plotted and show a remarkable resemblance to our data, demonstrating that, in fact, the observations on the dogs studied by Stabenau and associates agree with our observations on human beings. The only difference is in presentation of data and interpretation. For further clarification of this problem we can examine the report by Warren and Nathan[25] which deals directly with toxicity of ammonium salts and the ammonia concentrations found in the brains of mice. Table I of this paper shows little difference among the brain levels of ammonium produced by $L.D._{50}$ doses of acid or neutral ammonium salts, but there is a considerably lower level of ammonia in brain after an $L.D._{50}$ dose of ammonium carbonate, and the lowest level after ammonium hydroxide. This is remarkable, because if the death of the animals was caused by ammonium being forced into the brain because of alkaline pH, the brain levels should have been the same or higher than those found with an $L.D._{50}$ of acid salts. These data are compatible with the concept that alkalosis increases the lethality of ammonium, which was proposed by Roberts and her group,[17, 23] but not with the further proposition that this increased lethality is due to increased movement of ammonium into the brain. Table II of the paper by Warren and Nathan[25] also shows that severe alkalosis does not increase the level of ammonia in the brain, but actually decreases it. This is consistent with most of the data of the paper by Stabenau and associates, and if we look merely at the data without any preconceived notion about transport across membranes, we are forced to the conclusion that alkalosis does not drive ammonia into the brain.

It is obvious that tissue uptake is the product of blood flow and arteriovenous difference, but it requires a rather gymnastic approach to the data presented in this paper[25] and to that graphed by Stabenau and colleagues[20] to propose that the inverse relation seen between blood pH and arteriovenous difference may be counterbalanced at all times by a hypothetical alteration in blood flow. No conceivable alteration in blood flow could account for the change from positive to negative arteriovenous difference seen in our Figs. 1 and 2 and in Table I. These values were taken at close enough intervals that the inertia of the blood flow through the extremity or brain would not have given these results. For example, in Fig. 2 the very low arterial ammonia level persisted over a 10-minute period, and 2 consecutive observations, allowing even for a mean transit time of ammonia of 10 minutes through the extremity, clearly show this reversal of arteriovenous difference in direct relation to the arterial level. The data of Table I and Figs. 1 through 4 show little acute change in pH of blood passing through the tissues, although fairly wide ranges of pH were traversed. The pH and ammonia values appear to follow a regular pattern and do not oscillate significantly. All of the curves are relatively monophasic over fairly long periods. The parellel between arterial level and arteriovenous difference in all of the data must be given some weight.

The data of our Figs. 5 and 6 underline the above proposition, and show no correlation between arterial pH and arteriovenous difference, but a clear correlation between arterial level and arteriovenous difference. This is consistent with previous data from our laboratory and from that of Webster and Gabuzda.[26] It should also be noted that although Fazekas and his co-workers[9] are often quoted as showing no relation between arterial level and uptake of ammonia by brain, their actual conclusion is that in cirrhosis there is no greater uptake of ammonia by brain in comatose patients than in noncomatose patients. A plot of their data for arteriovenous differences versus arterial level of ammonia shows the same relation between blood level and cerebral arteriovenous difference that we have reported. Summerskill and associates[22] show that there

is occasionally no cerebral uptake of ammonia in hepatic coma. Nevertheless, their data also reveal the same general trend of increasing arteriovenous difference with arterial level.

The patients described in our report were under many different conditions of body temperature and were undergoing operation. The samples were taken at least 2 minutes after occlusion of the vessels was released, allowing time for "washout" of the circulation. Venous pH values had returned to preocclusion levels, which suggested a return of tissue pH to normal. The extreme tissue acidosis which must occur during occlusion[5] should stimulate tissue uptake of ammonia unless arterial pH drops remarkably. This phenomenon was not seen in the patients studied. It is the very point of our data, particularly of Figs. 5 and 6, that under a variety of circumstances the arteriovenous difference is proportional to the arterial level and is not related to the pH.

Let us interject at this point a plea for analysis of the data for its own sake rather than from the standpoint of a theoretical bias. There is no doubt that the pH of an ammonium solution influences the quantity of diffusible species present. This is no proof that the role of pH in movement of ammonia into tissues under physiologic conditions is more than trivial. Experiments in which animals are drowned in floods of intermediates do no more than demonstrate physicochemical phenomena, predictable from physical data. Their physiologic significance is questionable. The remarkable fact is that living organisms confound these experiments by demonstrating, frequently, disparate results. These cannot be tossed aside lightly, for they show that the physical principles invoked as mechanism are trivial in comparison to the physiologic phenomena which are at play. In regard to ammonium uptake by tissues, it is apparent that although pH may theoretically affect it significantly when the quantities involved are beyond the physiologic and pathologic range, it also appears that the pH effect is insignificant in proportion to the chemical mass action effect of arterial concentration.

SUMMARY AND CONCLUSIONS

Serial observations of arterial pH and arteriovenous difference of ammonia have been made on 11 patients undergoing hypothermia and cardiac operation over a prolonged period. The fluctuations in the arteriovenous differences of ammonia, both peripheral and cerebral, did not correspond to the fluctuations in pH, but corresponded remarkably closely to alterations in the arterial level of ammonia. It is therefore concluded that in a particular individual, the movement of ammonia into or out of the tissues is a function primarily of the arterial level of ammonia, rather than of the arterial or venous pH of the blood. An analysis of data reported from other laboratories also supports this view. An explanation of the experimental observations relating blood level of ammonia and pH can be obtained from the reported effects of pH upon the synthesis of urea.

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Mechanism of declamping shock in operations on the abdominal aorta

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A common problem encountered in the resection of abdominal aortic aneurysms is the marked drop in blood pressure which may occur when the clamps are removed.[1, 2, 4, 6] In our experience the magnitude of the drop is quite variable, ranging from 10 to 130 mm. Hg in 23 cases studied. The etiology of this pressure drop has been presumed to be a form of normovolemic shock in which blood is pooled transiently in the areas distal to the clamp.[3] An experimental study was designed to investigate this hypothesis and possible methods of prevention of the hypotension.

METHODS

Mongrel dogs were used in this study. The anesthesia employed was pentobarbital sodium, 30 mg. per kilogram. The dogs were divided into 2 groups.

Group 1. In 17 dogs the aorta just distal to the left renal vein was clamped for 1 hour. During the clamping period 8 animals received 200 ml. of whole blood; the remainder received an equivalent volume of 6 per cent dextran in isotonic sodium chloride.

Group 2. In 13 dogs the effects of duration of occlusion were observed by changing the clamping period to 15 minutes and subsequently to 1 to 2 minutes.

Four parameters were studied—central aortic pressure, cardiac output, leg volume changes, and femoral arterial pressures.

Central aortic pressure. A No. 205 polyethylene catheter was inserted into the aorta via the left brachial artery. Pressures were measured by a Statham pressure transducer and Sanborn Twin-Viso amplifier and recorder.

Cardiac output. Cardiac outputs were measured in animals in Group 1 before clamping, at the end of the clamping period, and within 1 to 2 minutes of declamping. To measure cardiac output, 20 to 50 μc of I^{131} was injected into the inferior vena cava. The sampling site was the central aorta. An automatic pipetting machine* was utilized which sampled 0.5 ml. per second and delivered this volume into a multiple fraction collector. Cardiac outputs were calculated from the obtained dye-dilution curve.

Leg volume changes. Changes in both foreleg and hindleg volume were monitored by a mercury strain gauge plethysmograph.[7] Particular attention was paid to the volume shifts which occurred with clamping and declamping.

Femoral arterial pressures. In Group 2 animals, femoral arterial pressures were monitored with a No. 90 polyethylene catheter inserted so as not to obstruct arterial inflow to the extremity.

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*Filamatic Duplex Automatic Pipetter, Model DAB, National Instrument Company, Baltimore, Md.

After base-line responses were obtained on animals in Group 2, two studies were performed to modify or eliminate the pressure drop. (1) In 8 animals, 60 to 320 mcg. of levarterenol* was injected distal to the clamp immediately prior to declamping. (2) In 5 animals, 60 to 240 mcg. of levarterenol was injected proximal to the clamp into the central arterial circulation prior to declamping.

RESULTS

Group 1. When the aorta was clamped just distal to the left renal vein there was an immediate increase in the average mean pressure from 101 to 109 mm. Hg, a 7.4 per cent rise. During the hour of clamping the mean pressure gradually increased to 123 mm. Hg, a 17 per cent rise from base line. This increase was probably the result of the 200 ml. infusion of whole blood or dextran during this time.

When the clamp was removed the mean aortic pressure dropped an average of 23 per cent, from 117 to 90 mm. Hg. There was essentially no difference in the animals which received whole blood and those which received the dextran. The cardiac output dropped an average of 21 per cent, from 1,350 to 1,060 ml. per minute when the clamp was removed (Table I).

The hindleg volume rapidly decreased, with aortic occlusion reaching a plateau within 30 seconds (Fig. 1). When the clamp was removed, leg volume increased and reached its maximal level in approximately 30 to 60 seconds. The leg volume then gradually decreased toward the preclamping values.

Group 2. In these animals the magnitude of the pressure drop did not vary significantly regardless of the duration of occlusion. The average gradient of pressure as reflected in the difference between aortic and femoral arterial pressure across the clamp at the time of declamping was also essentially the same.

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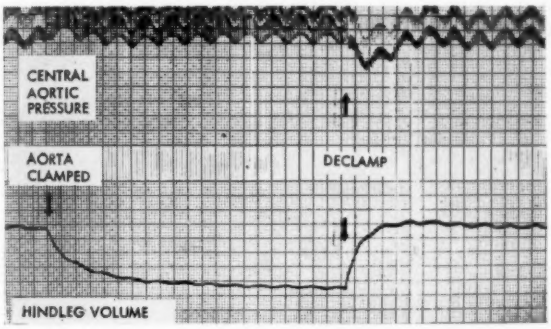

Fig. 1. Plethysmographic record which shows rapid decrease in hindleg volume with aortic clamping. Acute increase in leg volume with declamping coincides with pressure drop.

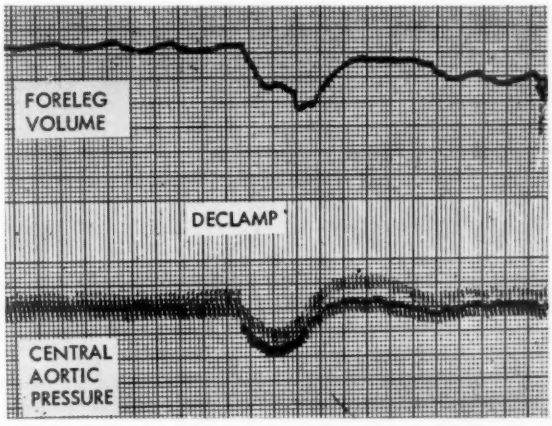

Fig. 2. Foreleg volume decreases simultaneously with drop in central aortic pressure at declamping.

The hindleg volume changes were the same as those noted in Group 1. The displacement of upper extremity blood volume with aortic declamping coincided almost exactly with the central aortic pressure drop (Fig. 2). As the central blood volume is translocated, the foreleg volume decreases. Thus, an inverse relation between foreleg and hindleg volume changes was observed at the time of declamping (Fig. 3).

The rapid injection of levarterenol distal to the clamp prior to removal was the most effective measure employed in reducing or eliminating the pressure drop (Table II). The clamp was removed when the maximal femoral arterial pressure was attained. In the group with 15-minute occlusion, the pressure drop was reduced from an average

Table I. *Results of varying the duration of aortic occlusion*

Occlusion time	*Average mean aortic pressure (mm. Hg)*		*Average pressure gradient at declamping (mm. Hg)*	*Average cardiac output (ml./min.)*	
	Before declamping	*After declamping*		*Before declamping*	*After declamping*
1 to 2 min. (7 animals, 14 occlusions)	126	100 (21% decrease)	89	—	—
15 min. (13 animals, 13 occlusions)	139	107 (23% decrease)	91	—	—
1 hr. (17 animals, 17 occlusions)	117	90 (23% decrease)	—	1,350	1,060 (21% decrease)

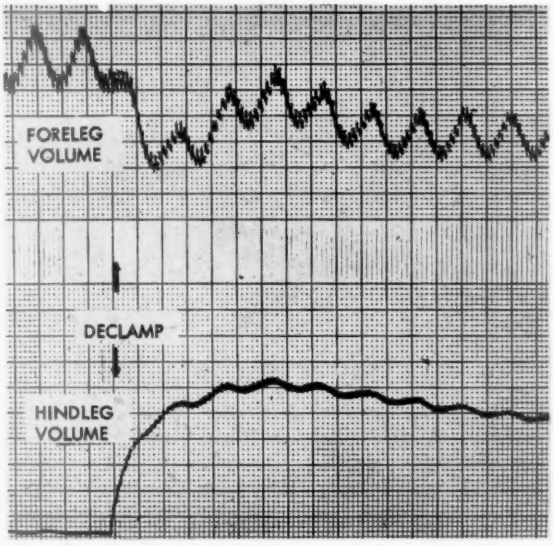

Fig. 3. Inverse relation between foreleg and hindleg volume at declamping.

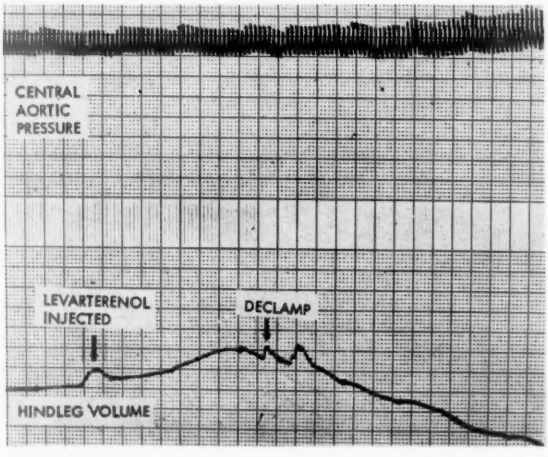

Fig. 4. Effect of 60 mcg. of levarterenol injected distal to clamp. Hindleg volume increases as blood pools on arterial side. When peak leg volume was reached, declamping resulted in no change in central aortic pressure.

of 23 to 5 per cent. In the group with 1- to 2-minute occlusion, the response was reduced from 21 to 8 per cent. In each instance the gradient of pressure across the clamp was greatly reduced.

When levarterenol was injected distal to the clamp, the increase in hindleg volume was greatly minimized or did not occur, depending upon the magnitude of the pressure drop (Fig. 4). If no drop in pressure occurred, no sudden increase in hindleg volume was recorded. Similarly, the decrease in foreleg volume was greatly reduced or abolished, depending on the amount of blood volume shift to the hindleg.

The rapid injection of levarterenol into the central arterial circulation proximal to the clamp did not alter the response to declamping in the 5 animals tested (Table II). The foreleg and hindleg volume shifts still occurred in these animals.

DISCUSSION

An average decrease in mean aortic pressure of 23 per cent after clamp release in animals subjected to 1 hour of aortic occlusion without significant blood loss suggests that this shock is indeed a redistribution phenomenon. The decrease in cardiac output (average 21 per cent) was accompanied by a corresponding increase in hindleg volume, which also suggests a normovolemic shift.

In the Group 2 animals the aortic pressure drops at 1- to 2-minute and 15-minute occlusion were essentially the same as drops for the 1-hour occlusion–21 and 23 per cent, respectively. The independence of pressure-

time relationship is apparent in these experiments.

The magnitude of the pressure drop appears to be related to the gradient of pressure across the clamp and the extremity blood volume at the time of declamping. Since the vascular bed is elastic, the greater the pressure head to which it is suddenly subjected upon declamping, the greater will be the volume displaced into it.

The most effective method employed to minimize or prevent the pressure drop was to inject levarterenol distal to the clamp immediately prior to declamping. Levarterenol infusion results in an increase in femoral arterial mean pressure and an initial increase in limb volume followed by a decrease. Shadle and associates[5] believe the initial volume increase is the result of arteriolar constriction which impounds blood on the arterial side. As arterial pressure increases and overcomes arteriolar resistance, an increased amount of drug is presented to the veins. This results in venous constriction which increases venous return and decreases the limb volume. This finding is substantiated by the plethysmographic data (Fig. 5). Thus, increased arteriolar resistance seems to be the most important factor in preventing the volume shift and subsequent hypotension, since the clamp may be removed before the venous constriction phase and the drop in pressure will not occur (Fig. 4). However, the vasopressor continues to be effective in preventing the volume shift and hypotension during the venous constriction phase (Fig. 6).

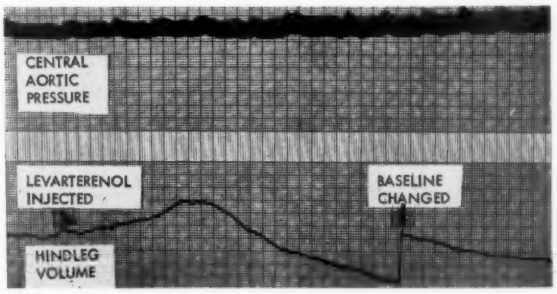

Fig. 5. After distal injection of levarterenol, hindleg volume increase is followed by rapid decrease as the vasopressor reaches the venules and veins, constricting them and increasing venous return.

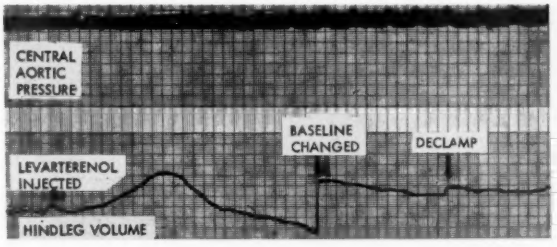

Fig. 6. Effect of vasopressor in preventing hypotension during venous constriction phase. Clamp removal was approximately 1 minute after hindleg volume began to decrease.

Any measure which tended to increase the central pressure alone or in greater proportion to the distal pressure would tend to increase the drop in pressure. Although the injection of levarterenol proximal to the aortic clamp did not increase the drop in

Table II. *Effect of levarterenol on the magnitude of the pressure drop*

Occlusion time	*Average central aortic mean pressure (mm. Hg)*		*Average pressure gradient at declamping (mm. Hg)*
	Before declamping	*After declamping*	
Levarterenol (60 to 320 mcg.) injected distal to clamp immediately prior to declamping			
1 to 2 min. (4 animals, 8 occlusions)	129	118 (8% decrease)	60
15 min. (7 animals, 7 occlusions)	139	133 (5% decrease)	53
Levarterenol (60 to 240 mcg.) injected proximal to clamp immediately prior to declamping			
1 to 2 min. (5 animals, 7 occlusions)	133	102 (23% decrease)	—

pressure, it certainly did not reduce it. The drop was not increased because the distal pressure was also elevated and tended to keep the gradient about the same.

There is one major difference between the experimental situation and that encountered clinically. In the clinical situation, there is frequently a fair amount of bleeding from the anastomoses. This will tend to add the problem of hypovolemic shock to the already existing redistributional shock.

SUMMARY

The acute drop in blood pressure which occurs at the end of temporary abdominal aortic occlusion is the result of translocation of blood into the vascular bed distal to the clamp and is independent of the duration of clamping if blood volume is unchanged. The gradient of pressure across the clamp at the time of release of occlusion appears to be the most important factor determining the magnitude of the blood volume shift and resultant drop in pressure.

The rapid injection of levarterenol distal to the clamp immediately prior to removal is effective in eliminating or minimizing the drop. This vasopressor drug rapidly raises arteriolar resistance and prevents displacement of blood to the distensible venous bed.

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Thrombogenic properties at the site of vascular anastomoses studied by a new technique for measuring platelet adherence

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The uninjured intimal surface of blood vessels is endowed with the property of preventing blood coagulation. However, when the intima is injured, it no longer retains this anticoagulative property, and the streaming blood tends to clot at the site of the injury. In this sense, the injured intima may be said to be predisposed toward thrombus formation. Many reports concerning the reparative process of the injured blood vessel wall have been published since the last century, but information bearing on the thrombogenic condition of the injured intima is very scant.

We have recently developed a new method for measuring the degree of predisposition of the intima toward thrombus formation.[3, 4] This involves measurement of the degree of adherence of radioactively labeled blood platelets at the surface of the intima. In the present series of experiments the method was applied to study changes in the state of the intima during the healing process at sites where vessels had been sutured or anastomosed. It was found that at such sites the risk of thrombosis is greatest on the fifth or sixth day after operation. The technique which was used to study these changes and the results of the experiments are discussed.

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EXPERIMENTAL METHOD

One hundred and forty-three dogs were used. After anesthesia (Orlopan given intravenously) had been induced, the abdomen was opened and the 4 operations were carried out, (1) end-to-end anastomosis of the transected abdominal aorta by Carrel's manual suture technique, (2) closure of the longitudinally slit abdominal aorta by continuous manual suture, (3) end-to-end anastomosis of the transected inferior vena cava by the Carrel technique, and (4) end-to-end anastomosis of the transected abdominal aorta with the suturing apparatus devised by one of us (K. I.).[5, 6]

When suturing was done by hand, No. 4-0 silk thread and an atraumatic needle were employed. No anticoagulants were used. After varying periods of time, the dogs were sacrificed and the vessel specimens were removed with approximately 2 cm. left on each side of the anastomotic site.

Canine blood platelets, separated by centrifugation, were suspended in physiologic saline solution, incubated with P^{32} for 2 hours at 37° C, according to the method of Julliard,[7] and washed with saline solution until no further decrease appeared in the radioactivity of the supernatant. The concentration of the platelets was adjusted to about 2 times 10^5 per cubic millimeter.

The specimen was cut open lengthwise

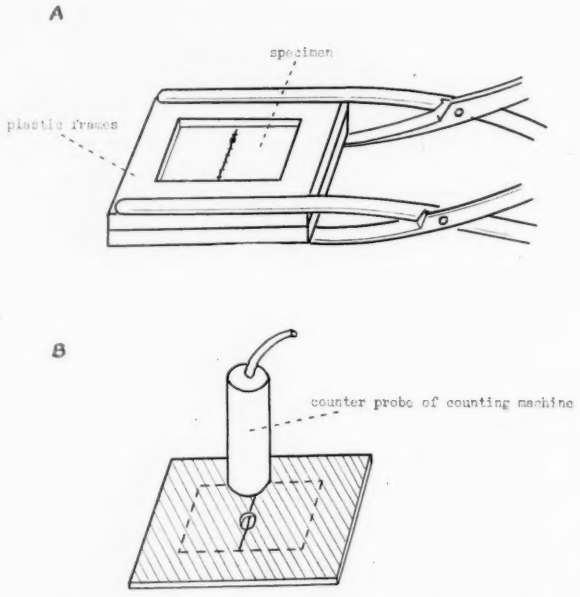

Fig. 1. Schematic illustration of procedure for measuring the adherence of radiolabeled platelets onto the intimal surface of blood vessel specimen. The vessel specimen is set in a pair of plastic frames and allowed to remain in contact with the radiolabeled platelet suspension for a measured period of time (*A*). The specimen is detached from the frames and the radioactivity on the intimal surface is measured (*B*).

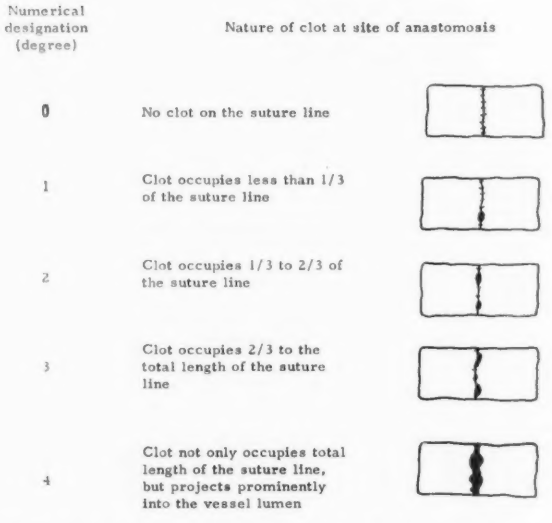
Numerical designation (degree)
Nature of clot at site of anastomosis
0
No clot on the suture line
1
Clot occupies less than 1/3 of the suture line
2
Clot occupies 1/3 to 2/3 of the suture line
3
Clot occupies 2/3 to the total length of the suture line
4
Clot not only occupies total length of the suture line, but projects prominently into the vessel lumen

Fig. 2. Arbitrary classification of the extent of clot attached to the anastomotic site. Degrees of clot attached to the suture line in Figs. 3 through 5 and 7 are taken as the average values of the numerical designation of each case.

and set in a pair of plastic frames as shown in Fig. 1. These frames, 3 mm. in thickness and with an opening of 3.0 by 0.9 cm., were so made that when fastened together with clamps, no leakage occurred at the lines of contact with the specimen. Thus, the intima of the specimen to be tested formed the bottom of a shallow tray. The seal of the tray was tested for 5 minutes with saline solution. Then, 2 ml. of the platelet suspension was poured into the tray, and allowed contact with the intimal surface for 5 minutes at room temperature. After this procedure, the tray was upset to throw away the platelet suspension, and rinsed in saline solution for 30 seconds. Then the specimen was detached from the frames and the degree of radioactivity on the intimal surface was measured with a Geiger-Müller counter. A lead shield with a circular opening 5 mm. in diameter was used for selecting the desired portion of the specimen. This was placed over the suture line at the point where there was the least amount of clotting. The same procedure was carried out on a segment of normal thoracic aorta from the same dog, and the level of radioactivity thus measured was used as a control value. The rate of adherence of blood platelets onto the intima was defined as:

$$R = \frac{I_a - N}{I_c - N}$$

R is the rate of adherence, I_a the counts per minute on the anastomosed portion, I_c the counts per minute on the normal intimal surface, and N the background counts per minute for the counting machine. The measurements were made 4 times at each site, and the average values were taken.

Attention was paid to the size of the clot adhering to the intima, and also to the extent to which the newly growing intima covered the anastomotic site on successive days after operation. Both gross and microscopic examinations were made. The arbitrary classification shown in Fig. 2 was used to indicate the size of the attached clot.

EXPERIMENTAL RESULTS

Group 1. End-to-end anastomosis of abdominal aorta with Carrel's method. Fifty-

two dogs were used in these experiments. Macroscopically, clot was seen at the anastomotic site in almost all the specimens from the first to the eighth postoperative day. The clot increased in amount gradually as time elapsed, and attained a maximum on the fifth day, while on the sixth to the eighth day the clot began to decrease. The clots shortly after operation were generally red or red brown with a smooth surface, while those after about the sixth postoperative day were dark red or brown with a somewhat rough surface.

After 9 days had elapsed, macroscopic observation showed a thin, glistening layer covering the suture site in almost all specimens, while 6 to 8 days after the operation the anastomotic line was seen to be covered by newly growing tissue in some specimens, but not in others. Macroscopic findings indicated that the development of intima began on about the sixth day and was complete on approximately the tenth day after operation.

New intima tended to develop more poorly over clotted than over clot-free surface. In specimens from approximately 2 weeks to 7 weeks after operation, the inner layer at the anastomotic site projected slightly above the surface of the surrounding intima. This disappeared by the fourteenth week.

It was seen that the platelet adherence rate on the clot-free surface, which was 4.8 on the first postoperative day, generally increased until it reached a maximum of 6.6 on about the fifth day and then decreased rather steeply to about 3.0 on the eighth to ninth day, gradually approaching the normal level after 100 days or more. Adherence at the surface of the clot followed a similar pattern, though the values in the latter case were approximately 15 per cent higher. These results are shown in Fig. 3.

Group 2. End-to-end anastomosis of inferior vena cava with Carrel's method. Thirty-one dogs were used in these experiments. Immediately after operation, the platelet adherence rate increased and reached a maximum of 7.2 on the sixth postoperative day. Thereafter the rate decreased sharply, leaving a marked peak in the curve. The size of the clot, still small

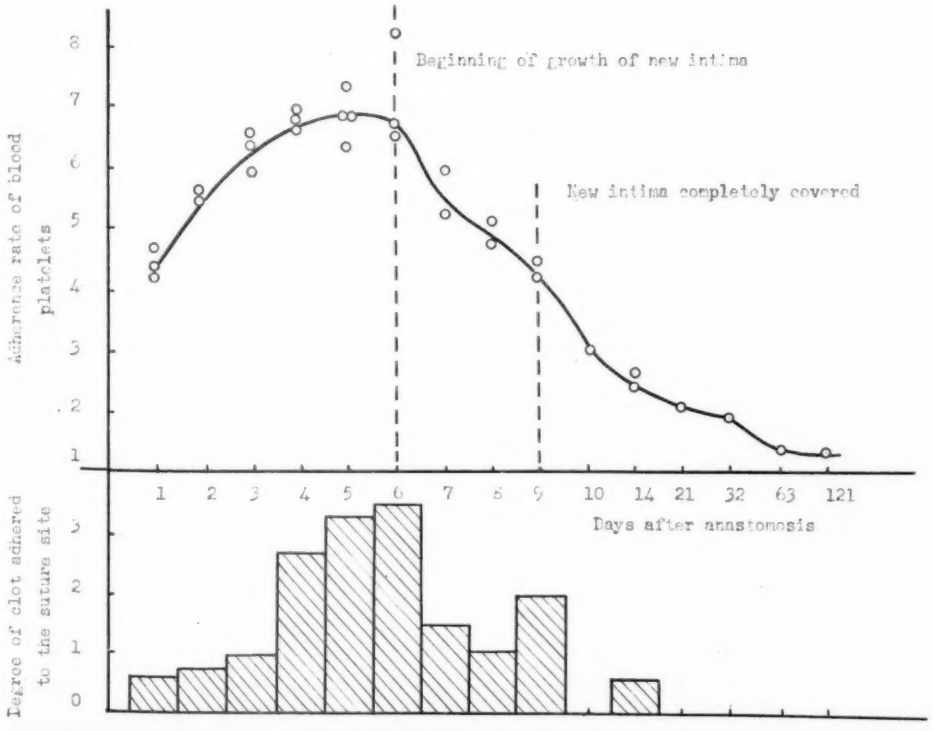
Beginning of growth of new intima
New intima completely covered
Adherence rate of blood platelets
Degree of clot adhered to the suture site
1 2 3 4 5 6 7 8 9 10 14 21 32 63 121
Days after anastomosis

Fig. 3. Platelet adherence rate at anastomotic site of abdominal aorta with Carrel's method

on the third postoperative day, increased rather sharply on the fourth to the sixth postoperative day, and then decreased again on the eighth to the ninth day. Intimal development was seen to begin on about the sixth day and was complete on the ninth day. The surface of the intima was rough in specimens excised during the first 30 days, while in those taken after 60 days it had become smooth and hardly distinguishable from the original intima. The behavior is shown in Fig. 4.

Group 3. Continuous hand suture of longitudinally slit abdominal aorta. Thirty-two dogs were used in these experiments. The platelet adherence curve, which began at 2.5 on the first postoperative day, increased steeply to 5.2 on the fourth day, and then decreased gradually to about 2.0 on the tenth day. The size of the clot attached to the anastomotic site was maximal on the fifth day, though the size was generally lower than in Groups 1 and 2. It was observed that the newly growing intima began to develop on the sixth postoperative day and completely covered the suture line on the ninth postoperative day. This is shown in Fig. 5.

Group 4. End-to-end anastomosis of abdominal aorta with the suturing apparatus. Twenty-eight dogs were used in this group. Only 3 specimens, taken on the fifth, seventh, and ninth days, showed clotting, and even in these cases the extent of the clot was small (1 degree or less). In other specimens, no clot was observed. Fig. 6 shows a comparison of the appearance of abdominal aorta segments anastomosed by apparatus and by hand. Whereas the specimen 4 days after being anastomosed by hand suture shows an appreciable clot, the mechanically sutured specimen showed very little clot. The platelet adherence rate, 2.3 on the first postoperative day, passes through a maximum of 3.5 on the fifth day, drops to 2.0 by the fourteenth day, and then gradually decreases to the normal level. It will be noticed that these values are only about one-third those obtained with Carrel's method. The newly growing intima was seen to begin to develop on the fifth postoperative day by manual suturing technique. With

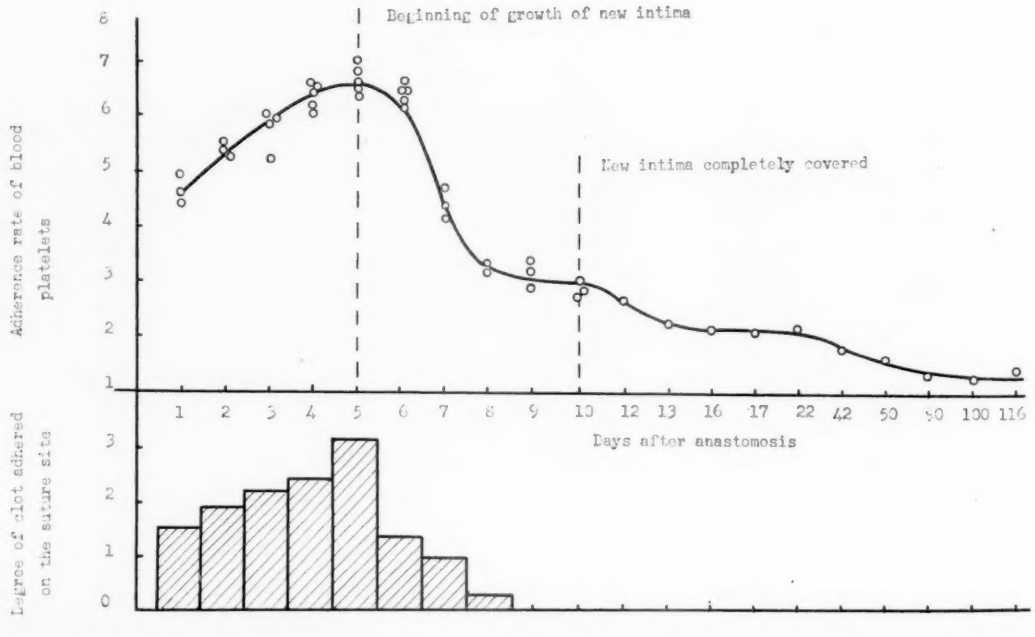
Beginning of growth of new intima
New intima completely covered
Adherence rate of blood platelets
Degree of clot adhered on the suture site
Days after anastomosis
1 2 3 4 5 6 7 8 9 10 12 13 16 17 22 42 50 90 100 116

Fig. 4. Platelet adherence rate at the anastomotic site of inferior vena cava with Carrel's hand suture method.

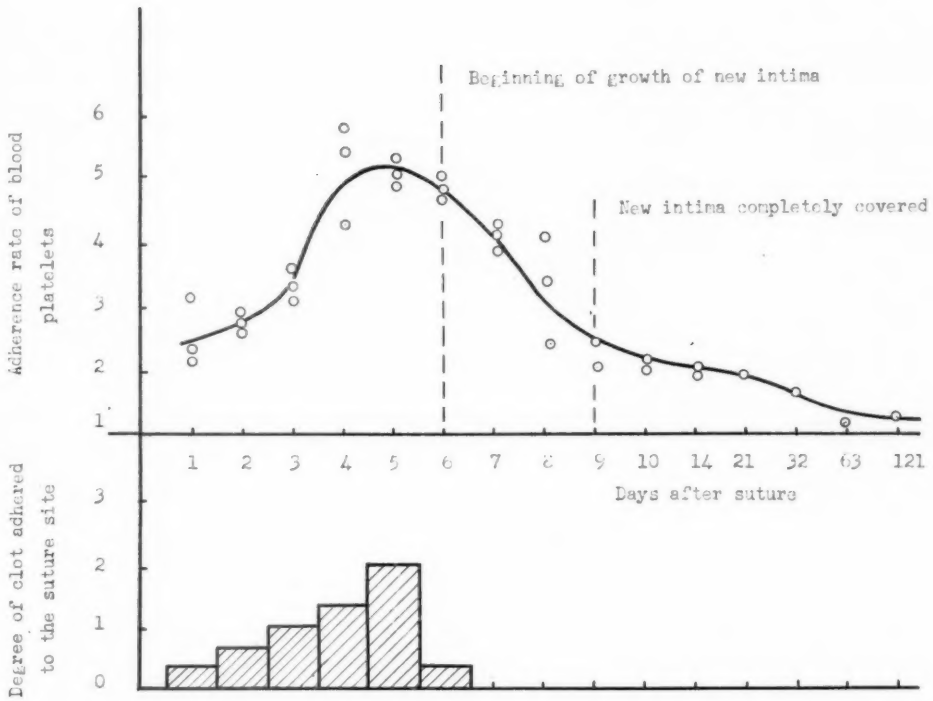
Beginning of growth of new intima
New intima completely covered
Adherence rate of blood platelets
Degree of clot adhered to the suture site
Days after suture
1 2 3 4 5 6 7 8 9 10 14 21 32 63 121

Fig. 5. Platelet adherence rate at the site of continuous hand suture of longitudinally slit abdominal aorta.

mechanical anastomosis, intimal formation began on about the seventh day, and was complete on approximately the tenth day. These results are shown in Fig. 7.

DISCUSSION

It generally has been recognized that the aggregation of blood platelets constitutes the initial stage in thrombus formation. Bizzozero[1] and Eberth and Schimmelbusch[2] reported that platelets in streaming blood tend to adhere to the injured portions of blood vessels. In addition, recent papers by O'Neil,[10] Moolton,[8] and Samuels[11] deal with the adherence of platelets to the injured intima. Previous studies at this laboratory[3, 4, 9, 12] have recently shown that radiolabeled platelets strongly adhered to the damaged intima of excised specimens of blood vessels as well as onto freshly clotted surface. Moreover, these experiments have shown that the adherence of platelets takes place in advance of macroscopic evidence of thrombosis.

In the present study, platelet adherence at the anastomosed or sutured site was studied on successive days after operation. Significant correlation between clot size and the platelet adherence rate at the clot-free surface was found. These facts lead us to conclude that the rate of platelet adherence at the intimal surface may be a good index

Fig. 6. Comparison of the intimal surface of anastomotic sites of dog's abdominal aorta with mechanical suture *(A)* and hand suture *(B)*. No clot with mechanical suture is seen.

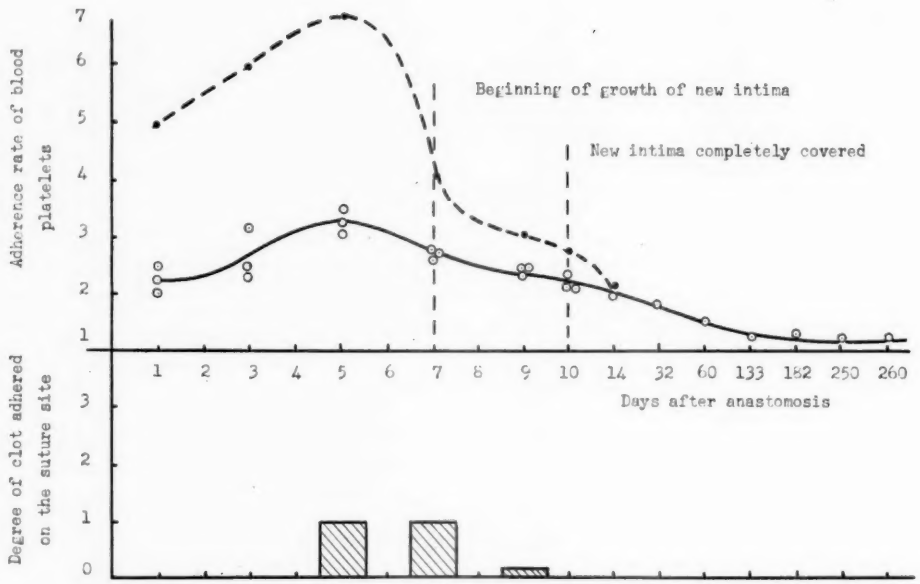
Adherence rate of blood platelets
Beginning of growth of new intima
New intima completely covered
1 2 3 4 5 6 7 8 9 10 14 32 60 133 182 250 260
Days after anastomosis
Degree of clot adhered on the suture site

Fig. 7. Platelet adherence rate at the anastomotic site of abdominal aorta with Inokuchi's mechanical suturing apparatus (solid line). Broken line indicates the rate by hand suture. Apparent low values with the mechanical suture are noted.

for the evaluation of the predisposition of the intima toward clot formation.

In the specimens in the present study, peak adherence of platelets occurred on approximately the fifth postoperative day. This was true regardless of the type of vessel anastomosed (abdominal aorta or inferior vena cava), or the technique used (manual or mechanical), or the kind of anastomosis performed (end-to-end anastomosis or closure of longitudinal slit in vessel wall). In all cases the extent of the clot was also greatest on about the sixth postoperative day. This seems to indicate that the intimal surface at the anastomotic site is temporarily especially prone to thrombus formation on about the fifth postoperative day.

It would be interesting to know how long it takes for the intima to regain its normal condition. In these experiments it took 2 to 3 weeks for the platelet adherence rate to fall to twice the control level and more than 10 weeks for it to drop to control levels. It is difficult to determine the exact stage at which the condition of the intima is no longer conducive to thrombus formation because the actual development of a thrombus depends not only on the condition of the intima but also on such secondary factors as vessel size, speed of blood flow, coagulability of blood, and so forth. However, it is clear that the condition of the intima is most favorable for thrombus formation on about the fifth postoperative day and that it takes some weeks for it to return to normal.

Dates of initiation and completion of new intimal development (determined by macroscopic inspection of the suture line) varied little with different types of experiments and were sixth and ninth days for the longitudinally slit abdominal aorta closed with a continuous hand suture, fifth and tenth days for the severed abdominal aorta anastomosed by Carrel's hand suture method, sixth and ninth days for the inferior vena cava anastomosed by Carrel's method, and seventh and tenth days for the abdominal aorta anastomosed mechanically. The period of development of intima corresponds to the decreasing slope of the platelet adherence curve, which falls to about 3.0 after new intima has covered the suture line. It will be seen that new intima begins to develop at a time when platelet adherence is close to its peak value.

In the case of anastomoses with Inokuchi's

suturing apparatus, there is much less platelet adherence and the clot at the anastomotic site is much smaller. It is apparent that this apparatus minimizes the risk of thrombosis after operation. It should be a particularly desirable instrument in anastomosing vessels of small caliber and veins, in which the difficulties inherent in manual technique ordinarily produce a high incidence of thrombosis.

Finally, it should be noted that our results have been obtained after operations on normal vessels in animals. In diseased vessels in human beings, the course of the reparative process may be somewhat different. The present paper, however, may be worthwhile in that it has demonstrated the basic pattern of the healing process from the standpoint of the thrombogenicity of blood vessels operated upon. Moreover, it seems to us that experimental methods of the type mentioned in this paper may yield still more information about the thrombogenic property of blood vessels than the studies reported here.

SUMMARY

In order to obtain greater understanding of the reparative process of the surface of the injured intima of blood vessels, anastomotic sites of abdominal aorta and inferior vena cava in dogs were studied by the technique of measuring the extent of adherence of P^{32}-labeled blood platelets onto the surface of the intima to be tested. The results obtained were: (1) platelet adherence was found to be related to the size of the clot and to the state of development of the intima at the anastomotic site, (2) platelet adherence at the anastomotic site reached a maximum on approximately the fifth postoperative day, and after the peak it took several weeks for degree of adherence to return to the normal preoperative level, (3) after anastomosis with Inokuchi's suturing apparatus, there was much less platelet adherence than after anastomosis by Carrel's manual suture method, (4) consequently, it might be deduced that the risk of thrombosis is greatest at about the fifth postoperative day, and that this risk can be minimized by use of the suturing apparatus.

The authors wish to express their thanks to Prof. M. Tomoda for his helpful encouragement throughout this work.

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The removal of a wire lodged in the interventricular septum of the heart

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The widespread use of the rotary lawn mower has resulted not only in direct injuries, but also in more serious trauma from missiles hurled by the whirling blade. This report concerns such an injury by a fragment of heavy wire 18 mm. long which penetrated the heart and lodged in the interventricular septum. A problem of accurate location of the foreign body, a veritable "needle in the haystack," arose since this was a prerequisite for its successful removal. It is the purpose of this report to describe the indications for the removal of such a foreign body, to restate the value of angiocardiography, to emphasize the use of temporary cardiopulmonary bypass of the heart to provide a relatively dry field for the removal of a small foreign body, and to call attention to the use of the Berman Locator.

CASE REPORT

G. D. H., a 30-year-old man, while mowing his lawn with a rotary mower on May 13, 1960, heard a loud explosion and felt a sudden pain in his chest. He was able to walk to his car, but fainted while on the way to see his physician, Dr. Frank Rainone. He soon regained consciousness and although he was hypotensive at first, his blood pressure soon returned to a normal level. He was hospitalized at the Flow Memorial Hospital in Denton, Texas, and was given intravenous fluids, antibiotics, and tetanus toxoid. The chest pain persisted. Roentgenograms of the chest showed what appeared to be a piece of bailing wire present in the region of the right ventricle. He was transferred to Baylor University Medical Center in Dallas on May 14, 1960, for removal of the foreign body. The wire had entered the chest in the fifth intercostal space, 4 cm. to the right of the midline, and the opening appeared to be 8 mm. in diameter. There were some nonspecific changes in the electrocardiogram which were definitely abnormal. Roentgenograms of the chest taken after a barium swallow showed the wire to be anterior, in the midline, and probably in the right ventricle projecting from the interventricular septum. On fluoroscopy, the wire was seen to move with each heartbeat, and appeared to be imbedded in the septum with the other end moving freely in the right ventricle. Angiocardiography confirmed the location (Figs. 1 and 2).

The patient was operated upon on May 19, 1960, through a median sternotomy after a roentgenogram of the chest had been taken on the operating table to be certain the foreign body had not moved. One hundred fifty milliliters of old and fresh liquid blood was found on opening the pericardium and there appeared to have been no clotting. The wire had entered the right ventricle anteriorly and inferiorly near the atrioventricular groove without injury to the right coronary artery and the point of entry was covered with a fibrin plug. The heart was examined digitally through the right atrial appendage, but the wire could not be felt. It seemed unwise to pursue this manipulation energetically for fear of dislodging the foreign body. The patient was prepared for total cardiopulmonary bypass with a Pemco Kay-Cross pump-oxygenator by administration of heparin and insertion of catheters into the right femoral artery and into the vena cava for siphon drainage. The Berman Locator was then used and although it appeared to locate the wire in the right ventricle, it gave a false reaction when in the metallic field of the sternal rib spreader. Upon opening of the right ventricle and evacuation of the blood, the foreign body, covered with fibrin, could be seen projecting from the interventricular septum, penetrating one of the chordae tendineae of the septal leaflet of the tricuspid valve. The wire was grasped and removed, leaving no apparent defect in the septum, and the ventriculotomy closed. The vessels were decannulated after 15 minutes of bypass and the cardiac activity remained vigorous and effective. The pericardium was closed loosely, chest tubes

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inserted for drainage, and the sternum approximated with stainless steel wire. The femoral and brachial arteries were repaired.

This patient's recovery after operation was excellent. He was given antibiotics and digitalized. The chest tubes were removed by March 22, 1960, and roentgenograms of the chest showed good lung expansion. The incision was healing well and the sutures were removed on June 1, 1960, and the patient discharged. The patient was last seen Oct. 17, 1960, after having gone back to work in August, 1960. His blood pressure was 120/80, no cardiac murmurs could be heard, and his condition is excellent with a complete recovery.

DISCUSSION

Foreign bodies may enter the heart directly or from a vessel proximal to a heart chamber. If the patient recovers from the initial injury, the problem is what to do with a patient who may or may not have symptoms and is discovered to have a retained metallic foreign body in the wall or chamber of the heart. The arguments advanced for the removal of cardiac foreign bodies[4] are (1) to prevent embolism of the foreign body or an associated clot, (2) to reduce the danger of bacterial endocarditis, (3) to prevent recurrent pericardial effusions, and (4) to diminish the incidence of myocardial damage with ventricular aneurysm and rupture.

On the other hand, many patients with foreign bodies in the heart have been described who were essentially asymptomatic for periods varying from months to years after their intial injury. Not everyone agrees that the hazards of retained foreign bodies are great. Fritz and associates,[3] performed experimental studies of foreign bodies in 62 dogs by introducing foreign bodies directly into the heart. They concluded that the majority of foreign bodies retained in and around the heart, as long as they do not produce symptoms or signs of cardiac dysfunction, may be permitted to remain undisturbed. However, a large number introduced directly into the chamber embolized unless they were caught in the chordae. It appears that foreign bodies may behave differently depending upon their location within the heart. Foreign bodies free in a chamber have a strong tendency for early embolism. Those entirely within the myocardium, which are not infected, will soon become enclosed in a fibrous capsule and will not tend to migrate or injure adjacent myocardium, while those which are in such a position that the myocardium or valve leaflet impinges upon them during cardiac systole tend to cause erosion and produce bacterial invasion and myocardial obstruction.[6] Decker,[1] after a review of 109 cases, stated that foreign bodies should not be removed simply because of their presence. "No emergency exists in those patients

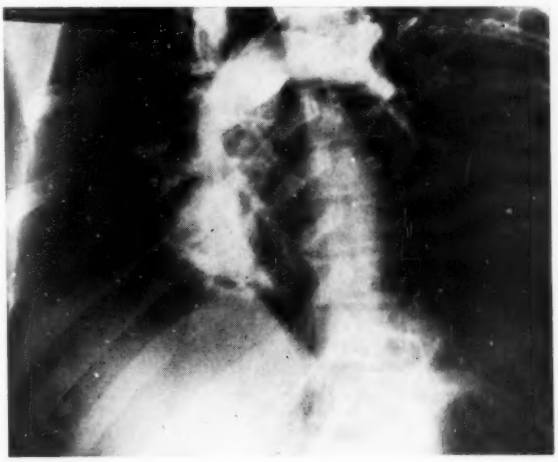

Fig. 1. Left anterior oblique angiocardiogram which shows the foreign body in the chamber of the right ventricle.

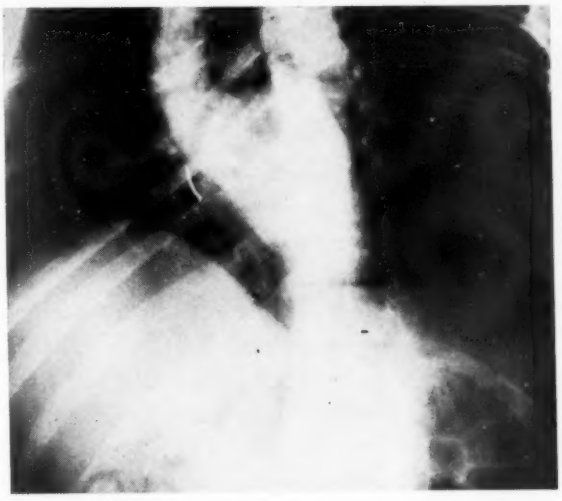

Fig. 2. Left anterior oblique angiocardiogram which shows the foreign body outside the cavity of the left ventricle with one end in the interventricular septum.

who survive the original trauma, unless one chooses to consider that free cavity bodies, sharp needle-like bodies and large objects need immediate or early surgery." The prominent subjective symptoms in these cases were pain, dyspnea on exertion, asthenia, and neurotic manifestations. In 2 of the cases reviewed the patients had involvement of the interventricular septum. This appears to be the rarest location. Magnan, in 1911, reported a needle three-fourths of an inch long in the interventricular septum which had been present 29 years and was not removed, but found at autopsy.[1]

Partial review of the literature indicates several deliberate attempts to remove foreign bodies in the interventricular septum. Swan and his group,[6] in 1952, reported 2 cases of foreign bodies in the interventricular septum. One, 1 cm. by 4 mm. in size, was wholly within the septum and there appeared to be no indication for its removal. The second was a shell fragment which was partially imbedded in the midpoint of the interventricular septum and protruded into the chamber of the right ventricle. There was pericardial effusion. Electrocardiogram showed a pattern of pericarditis with a right bundle branch block. The fragment was removed through an anterior bilateral sternum—dividing thoracotomy during inflow vena caval occlusion of 1 minute and 40 seconds through a right ventriculotomy. Valle,[7] in 1955, reviewed 117 injuries of the heart and pericardium which occurred in 2,811 casualties of the Korean conflict listed at the Tokyo Army Hospital. Nineteen had foreign bodies in the myocardium and these were removed successfully by closed methods except in 3 cases in which the foreign bodies were lodged in the interventricular septum. Efforts to remove these were unsuccessful. Flick,[2] in 1960, reported the removal of an embolic .22 caliber bullet trapped beneath the chordae tendineae of the tricuspid valve within the right ventricle. This was successfully accomplished in a 9-year-old boy by a closed method through the right ventricular wall. However, the foreign body could be palpated through the intact wall of the right ventricle.

The presence of a foreign body imbedded in the heart presents a difficult problem. The danger of allowing it to remain with the attendent consequences must be balanced against the hazard of removal. In the present report, the principal indications for the removal of the wire were the danger of bacterial endocarditis, embolization, and possible injury to the interventricular septum if the foreign body were allowed to remain. The patient's desire to be rid of a foreign body in his heart is of considerable psychological importance, but this presents no real indication for operation. Once the decision is made to remove the foreign body every effort should be made to locate it so that no time will be lost in seeking it. Cardiac fluoroscopy and angiocardiography should be used. Angiocardiography in the left anterior oblique view which outlines the interventricular septum is best for location of missiles in or near the ventricles. This is demonstrated by Figs. 1 and 2 in which it will be noted that the wire is outside the lumen of the left ventricle, but is in the region of the right ventricle. This was first emphasized by Swan and associates.[6] The foreign body in the interventricular septum is best removed during total cardiopulmonary bypass and the hazard of the cardiotomy in the otherwise healthy heart is considered to be minimal. This allows a relatively dry field and time for deliberate location and removal of the foreign body. The Berman Locator, which was successfully utilized by Simpson[5] in locating a foreign body in the sulcus between the right atrium and the right ventricle just to the left of the right coronary artery, emits a high-pitched noise when near metal, and was employed in this case. Although it appeared to locate the wire in the right ventricle, a false reaction was given when the probe was in the field of the sternum-splitting retractor. It seems advisable to remove all metallic objects from the immediate field if the use of the locator becomes critical.

SUMMARY

The successful removal of a fragment of baling wire which, propelled by a rotary

lawn mower, had penetrated the heart and lodged in the interventricular septum with one end free in the right ventricle is reported. This was accomplished during total cardiopulmonary bypass. The indications for the removal of foreign bodies located in the interventricular septum are reviewed. The accurate location by roentgenograms, fluoroscopy, and angiocardiography is stressed as a requirement for the successful removal of small foreign bodies in the heart.

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Gelatin sealing to prevent blood loss from knitted arterial grafts

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Early use of fabric arterial grafts was attended by serious hemorrhage when flow was re-established after surgical placement. Efforts to control blood loss by reducing pore size have been successful but as pore size has become smaller, fabric grafts have tended to resemble solid-wall tubes. Edwards[3] showed in 1957 that tubes with solid walls were unsuitable for use as arterial grafts because the solid walls delayed or prevented healing of the inner fibrin lining and thrombosis occurred frequently. After pondering the question of how to provide walls solid enough to be leakproof, yet porous enough to heal, we thought the answer might be a porous graft coated with an impervious material which would be slowly absorbed by the body. Gelatin film, known to be absorbable with minimal reaction, appeared to be a logical coating material. In the course of our experiments a flexible gelatin coating was developed and tested which controlled blood loss from fabric grafts while causing only a minimal delay in normal healing.

EXPERIMENTAL PROCEDURE

Sixty-nine arterial grafts, each measuring 5 cm. in length, were thoroughly impregnated with a gelatin solution. The experimental series consisted of 57 grafts of coarsely knitted Teflon, 9 grafts of coarsely knitted Dacron, and 3 grafts of braided nylon. A control graft of identical material was prepared for each gelatin-treated graft, giving a total of 138 grafts that were placed in animals.

The grafts were packaged in test tubes and each gelatin-coated graft paired with its control graft. The 69 pairs of grafts in their tubes were baked for 4 hours at 140° C. (284° F.) in a laboratory drying oven. The baked gelatin-coated grafts and their controls were then inserted into a series of mongrel dogs. The dogs were of small to moderate size, weighing an average of 14.5 kilograms. Most (42) of the pairs of treated and control grafts were used to bypass ligated segments of the right and left carotid arteries. Some (22) of the treated grafts were sewn end-to-end to their controls, and the paired grafts inserted to replace an excised segment of the abdominal aorta. The control graft was placed proximally in some animals and distally in others. A few femoral bypasses were attempted.

Each dog served as its own control and had treated and control grafts inserted into the same, or symmetrical, arterial sites on the same day. Animals were sacrificed at intervals and the grafts removed for evaluation. The grafts were examined, sketched, photographed, fixed in formalin, and sectioned. In an effort to overcome any bias, grafts were compared and evaluated before they were identified as gelatin treated or control.

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PREPARATION OF GELATINIZED PROSTHETIC GRAFTS

In this study the first attempts at sealing Teflon arterial grafts were made with commercially available Teflon tubing.* The grafts were one-fourth inch in diameter and were designated as 1040T knitted Teflon tubing. They were made of 400 denier, 60 filament yarn with 1 twist per inch and knitted at 60 courses per inch into crimped tubes. It was impossible to successfully coat this material with gelatin.

Close inspection of a graft showed that it had been partially sealed after knitting with Kel-F which prevented adherence of the gelatin. The manufacturer kindly provided uncoated grafts that were otherwise identical and these were readily sealed with gelatin. From this it was learned that all preliminary coatings on Teflon yarn must be avoided and gelatin must be applied directly to the raw fabric.

The Teflon grafts coated with Kel-F also had been treated by the calendering process whereby pore size was partially reduced. The flattening of the yarn during calendering may also have interfered with the bonding of the gelatin. Gelatin coating probably will be most successful on grafts that retain their original porosity as they come from the knitting machine.

The raw gelatin used as the sealing solution was USP pigskin gelatin† taken from the stock of raw materials used in the manufacture of gelatin film (Gelfilm) and gelatin foam sponges (Gelfoam). After experimenting with solutions of various concentrations maintained at various temperatures, we found the most satisfactory preparation to be a 7 per cent gelatin solution warmed to 70° C. in a water bath. During saturation of the material with the gelatin all air bubbles had to be carefully worked out of the fabric. Excess gelatin solution was drained from the fabric. Care was necessary to avoid stripping the drained graft between the fingers or squeezing it in the hand, for this led to leaks. An experiment demonstrated that drying the grafts in room air at normal temperatures by suspending them with a mild amount of weight on one end prevented adherence of adjacent ridges of the crimped fabric. This controlled the leaks that had appeared after separation of adherent ridges on the early gelatin-coated grafts. The initial drying in room air is important because the subsequent baking process will convert a moist jelled coating back into a warm liquid that will drain free of the graft when it is placed in the oven.

Baking is an essential part of the preparation, for it denatures the gelatin to render it less soluble in warm blood and more resistant to absorption by the tissues. Baking was accomplished by placing the grafts in a laboratory drying oven for 4 hours at 140° C. (284° F.). Aluminum containers were found to turn the grafts a very unattractive dark gray color during baking. Glass and some paper containers are more satisfactory. The baking process renders the grafts sterile and they can be maintained in this condition by storage in the closed containers in which they were baked. However, sterilization immediately prior to implantation in the animal or patient can be accomplished also by autoclaving for 5 minutes at 140° C.

Rough handling of the dry or baked grafts will crack the gelatin seal. Once the graft is remoistened, however, the gelatin coating becomes flexible and the graft can be handled freely at the operating table. It should be noted that excessive handling in a rough manner as well as sharp crimping of the material may lead to breaks in the continuity of the film and an unsatisfactory result.

As one extreme test of the sealing properties of gelatin a transparent gelatin tube was prepared by dipping a cylinder of nylon net into gelatin solution. The net openings were 2 mm. in diameter. The tube was used to replace a segment of aorta. Defective areas in the transparent tube began to leak within less than 1 minute, but it appeared that

*Furnished by the United States Catheter & Instrument Corp., Glens Falls, N. Y.

†Furnished by The Upjohn Company, Kalamazoo, Mich.

elimination of bubbles in the gelatin film might have given a stable and durable preparation. Such a tube of gelatin, supported by fabric net, might be used in experiments to help define the effect of variable porosity on graft healing.

RESULTS

Blood loss. The gelatin coating successfully controlled blood loss. In 77 per cent of the graft pairs the blood loss from the coated graft was prevented completely or reduced to a minimal ooze. Table I shows the blood loss experience. In every instance blood loss was less severe from the gelatin-coated graft than from the untreated control. While this data was being accumulated various concentrations of gelatin and various methods of preparation were being tried. If only the perfected preparations were included, the data would be even more conclusive. On the basis of this experience it appears that blood loss through fabric graft material which is quite porous can be completely prevented by gelatin treatment of the fabric.

The terms "heavy" and "minimal" used in Table I are relative and require further definition. Attempts to measured blood loss by weighing sponges were unsuccessful. Minor errors in fashioning the anastomosis had a greater influence on the blood loss than did the presence or absence of a gelatin coating on the graft—though gelatin-treated grafts in general showed less leakage along the suture line. An acute experiment was performed which helped to define degrees of blood loss.

The femoral artery of a dog was divided and plastic catheters were tied into the proximal and distal segments of the vessel. The catheter ends were joined by a 5 cm. segment of the graft being tested. Femoral flow was re-established via these catheters through the graft. Blood loss through graft pores was measured. Imperfect though they were, early gelatin coatings permitted total losses of only 50 and 75 ml. when, with no intermittent reclamping, these imperfectly coated grafts were made a part of the femoral circulation. This loss was characterized as "moderate." Loss through the control grafts was so severe under similar circumstances (700 ml.) that the dog went into shock and would have died of exsanguination had the clamps not been reapplied. This and similar losses were characterized as "heavy."

Table I. *Blood loss from arterial grafts*

	Gelatin coated		*Control*	
	No.	*%*	*No.*	*%*
Heavy	1	1	52	75
Moderate	15	22	17	25
Minimal	26	38	0	0
None	27	39	0	0

Thrombosis. There was no evidence that gelatin coating increased or enhanced thrombosis of grafts. The knowledge that gelatin foam sponges are in common use as hemostatic agents suggested that gelatin might have thrombogenic properties and be unsuitable as a material to be applied to vascular grafts. An acute experiment was set up to investigate the possibility.

Test tubes for the performance of Lee-White clotting time determinations were carefully cleaned. The determinations were then performed in control tubes and in tubes that had previously been coated with silicone, paraffin, and baked gelatin. As Table II shows, a baked gelatin lining was nearly as effective as a silicone lining in delaying clotting. This demonstrated that gelatin is not thrombogenic in vitro.

The results of animal experiments confirmed the impression that a gelatin film is not thrombogenic. Grafts that did clot in dogs were divided into (1) grafts that thrombosed within the first 24 hours after implantation and (2) grafts that thrombosed later in the postoperative course. This was considered an important division on a theoretical basis for, as Harrison[5] and Szilagyi[10] have pointed out, early thrombosis is often a result of shortcomings in operative technique while later thrombosis usually reflects undesirable characteristics of the graft material. All grafts which showed evidence of gross infection were excluded from this portion of the study.

With regard to the acute thrombosis encountered in carotid grafts (that is, thrombosis more likely to be due to poor operative technique) the results in the 2 groups were identical. There were 3 acute failures in 50 gelatin-coated grafts and 3 acute failures in 50 control grafts.

Late thrombosis (that is, thrombosis more apt to be influenced by the presence of a gelatin coating) was also as frequent among control grafts as among gelatin-coated grafts. In the 26 pairs of carotid grafts in which valid comparisons could be made, 3 gelatin-coated grafts and 3 control grafts showed late thrombosis. (Excluded from consideration were 27 animals with grafts other than carotid, 6 animals with carotid grafts that clotted within one day, 4 animals sacrificed on the first postoperative day, 4 animals with infected grafts, and 2 in which there had been technical errors.)

This experience with late thrombosis is of critical importance for 2 reasons. First, it establishes gelatin as a sealing material that can be used without fear of increased thrombosis. Second, this experience demonstrates that dog carotid arteries or other such vessels averaging 4 mm. in diameter can be successfully bypassed by fabric arterial grafts. Previous workers have abandoned carotid arteries as a useful implantation site because of thrombosis rates approaching 100 per cent. The success obtained in this experiment is probably due to the use of end-to-side anastomoses, careful operative technique, and the use of seamless tubes.

The similarity of treated and control grafts in their tendency to form thrombi is further evidenced by the 6 carotid grafts which became thrombosed 24 hours or more postoperatively. Bilateral thrombosis of carotid grafts in 2 dogs accounted for 4 of the 6 thrombosed grafts (2 gelatin-coated and 2 control grafts). Two other dogs developed late thrombosis in 1 of the 2 carotid grafts, 1 in a treated graft and 1 in a control graft. In both dogs the lining of the patent graft, opposite the thrombosed graft, consisted of unusually heavy fibrin which partially occluded the lumen. These linings were much heavier than those found in any other patent grafts in the series. Thus in each of the 6 instances of late thrombosis in carotid grafts there was evidence of a similar excessive fibrin deposit in both treated and control grafts. This indicates that the presence or absence of the gelatin coating was a factor of less magnitude in graft failure than the characteristics of the animal which received the grafts.

Table II. *Lee-White clotting time determinations (venous dog blood)*

Tube lining	*Determinations*	*Average (min.)*	*Range (min.)*
Clean glass	4	10.0	7.5-11.5
Paraffin	2	10.7	10.0-11.5
Gelatin	4	14.7	14.0-15.5
Silicone	2	16.5	13.5-19.5

Infection. In the 53 pairs of grafts which were valid for comparison with respect to infection, 75 per cent of the treated grafts were free of infection and 70 per cent of the control grafts were free of infection. Though treated grafts appear to be slightly superior to the controls, the difference was not significant.

Hematoma formation. Delayed leakage with hematoma formation about the graft was not a problem with either the treated or the control grafts.

Fibrin accumulation. Teflon grafts were selected for their property of accumulating only minimal amounts of fibrin—a property emphasized by Harrison[5] and Edwards.[4] It was found that these minimal accumulations were slightly increased by the application of a gelatin sealing coat. On the uneven surface of a crimped Teflon graft with minimal fibrin, the true thickness of fibrin is difficult to measure. By approximation it seemed that gelatin treatment increased the fibrin coat to 0.85 or 0.9 mm. in experimental grafts as compared to an average of 0.8 mm. in control grafts. However, variations in fibrin thickness from animal to animal were greater than the variations in fibrin thickness between treated and control grafts. It should be noted that, while the small in-

Fig. 1. Aortic graft. The horizontal arrow indicates the suture line joining the gelatin-coated segment on the left to the control segment on the right. The control segment is occluded by a solid fibrin plug which is sharply confined to that segment.

crease in fibrin in treated grafts is reported for the sake of completeness and accuracy, the increase was of no final importance since it was quite small and it did not lead to an increased thrombosis rate in gelatin coated grafts.

Results with aortic grafts indicated that the gelatin coating may actually retard or prevent heavy fibrin accumulations in some instances. There is no statistical evidence of this in the series, but close inspection of 3 aortic grafts that did thrombose suggested this conclusion. Recall that each abdominal aortic replacement graft consisted of a segment of gelatin-treated graft sewed end-to-end to its control graft. In some replacements the treated graft was placed distally and in others it was placed proximally. In Fig. 1 an aortic graft is shown which was occluded by a short cylinder of firm, pink fibrin. The fibrin had formed within the control graft only. By contrast, the treated segment had no fibrin plug but only a smooth, thin, 1 mm. lining of fibrin. Two other thrombosed aortic grafts showed a similar but less striking picture. They both had a pink clot in the control segment and a dark red clot in the gelatin-coated segment of the graft. The pink clots in the control segment were presumably formed by slow accumulation on the graft walls of fibrin in which very few red cells were trapped. When this fibrin in the control segment became thick enough, it slowed and finally stopped blood flow through the grafts. Blood in the adjacent gelatin-coated segment and the distal aorta then clotted abruptly with entrapment of red cells and formation of the uniform dark red clot. Thus, it seemed that the control segments and the pink clots found within them were responsible for thrombosis of the aorta and for the formation of the fresh dark red clots within the treated segments.

In the aortic grafts described, some process which was responsible for slow fibrin accumulation on the walls of control segments seems to have been repressed or inhibited in the gelatin-coated segments. Almost immediately after re-establishment of blood flow both treated and control segments of each of these aortic grafts must have been coated with a thin layer of fibrin deposited out of the flowing blood. From that moment on the physical and chemical characteristics of the linings of the treated and control segments should have been the same. Yet through some mechanism that continued to exert its effect through the thin fibrin barriers, the control segment accumulated fibrin to the point of occlusion while the fibrin deposits in the treated segment remained thin. The key to a fundamental understanding of processes at work in grafts that become thrombosed might lie hidden in these 3 aortic grafts. It could be that orientation of fibrin molecules absorbed into the gelatin, with respect to the electrical charges such fibrin molecules present to the moving stream of blood, might be a factor. The interested reader is referred to an excellent article by Sawyer and associates[9] concerning the electrical fields which are found about damaged vessels and their influence upon the clotting patterns within those vessels.

Disappearance of gelatin. Gelatin was deposited on grafts in a thin layer only one twentieth to one fiftieth as thick as the graft fabric. In Teflon grafts the gelatin filled the major pores and coated the fabric but it did not work its way around each individual

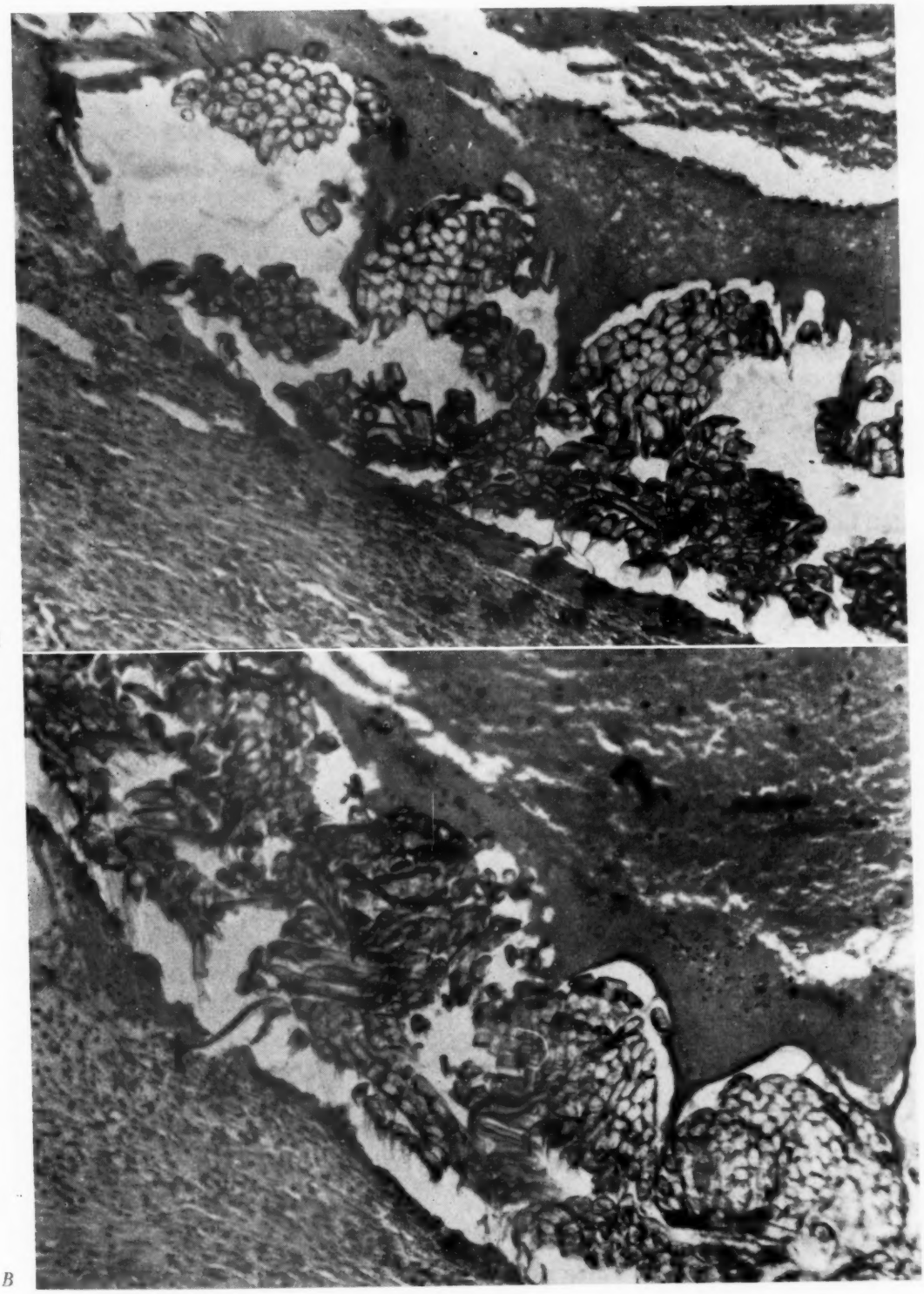

Fig. 2. A pair of Teflon carotid grafts which were removed on the twelfth postoperative day. *A*, The control graft. *B*, The graft coated with gelatin which appears as a dark line at the base of the fibrin lining of the graft. Note that there are nearly identical adventitial responses in the 2 specimens.

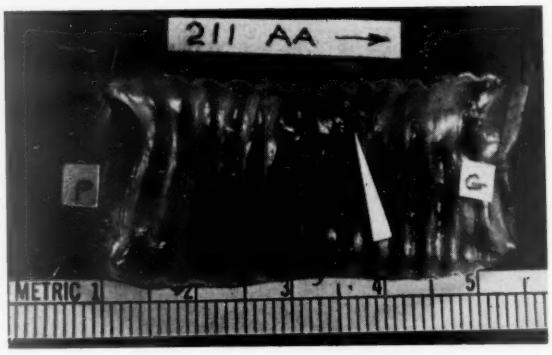

Fig. 3. An abdominal aortic graft removed 4½ months after implantation. The control graft is to the left (proximal) and the gelatin-coated graft is on the right. The arrow indicates a small area of minimal fibrin roughening still to be converted to fibrous tissue in the gelatin segment of the graft.

fiber. Such a gelatin coat appears in Fig. 2, *B*. On Dacron grafts the gelatin closed major pores but it also surrounded each fiber of the strands of yarn. This difference between the way Teflon and Dacron hold the gelatin coating seemed of no mechanical or biologic importance on gross or microscopic examination. Presumably the difference was due to the fact that Dacron fibers have a wettable surface and Teflon fibers have a nonwettable surface.

Teflon causes less reaction in surrounding tissue than any other graft material in common use. Teflon was selected for most of this experiment since tissue reaction was never severe enough to mask even mild adverse tissue reactions to gelatin.

In the experimental animals all traces of gelatin were gone completely by the twentieth postoperative day. Microscopic sections of a typical pair of grafts removed on the twelfth postoperative day are shown in Fig. 2. Tissue reactions about control grafts and gelatin-coated grafts are virtually identical grossly and microscopically at all stages of healing. Gelatin was absorbed by the body without accumulation of giant cells and with no greater number of macrophages or polymorphonuclear leukocytes present than were required to absorb and organize the fibrin deposited about control grafts. There was no evidence that the temporary presence of gelatin influenced in any way the amount of fibrous tissue deposited about the healed grafts.

Rate of healing. The gelatin coating did retard the rate of healing by 10 per cent. Early healing seems to begin by vigorous ingrowth of fibrous tissue and endothelium from the adjacent vessel ends. Early healing of this nature was not delayed by the gelatin coating.

Later healing takes place by fibrous replacement of the fibrin which lines the midportion of the graft. For this stage of healing it appeared necessary that fibroblasts grow in through the pores of the fabric. When gelatin temporarily sealed the pores, ingrowth of fibroblasts appeared to be slightly delayed. The delay amounted to 10 per cent of total healing time. Fig. 3 shows a pair of grafts removed 4½ months after implantation. Gross and microscopic patterns of healing in the gelatin-treated grafts and the control grafts are identical except for this slight delay in the healing time of treated grafts. Here again the differences between animals exceeded the differences in any one animal between a treated graft and its control.

DISCUSSION

Gelatin is believed to be a safe material for graft sealing. This conclusion is based on the demonstrated inertness of gelatin foam sponges which have been used clinically for years as hemostatic agents. Antigen-antibody reactions have not been a problem with the use of gelatin foam sponges or with repeated intravenous administration of gelatin.[7] It therefore appears unlikely that gelatin graft coatings would present any hazard to patients, even when used repeatedly.

From recent studies, the degree of porosity of vascular prosthetic grafts appears to be an important consideration in their ultimate successful use. Edwards[3] has shown the necessity for pores that will permit fibroblasts to grow into the lumen of the graft, organize the fibrin, and heal the graft in place. Best results are obtained when the

fibroblasts can penetrate the interstices of graft material into the inner fibrin lining from their source in the surrounding tissue. Other investigators[1, 2, 8] suspect that infections are a greater problem with impervious grafts than with porous grafts. However, substantial proof for this impression is lacking as yet.

Coincident with the need for an as yet unspecified degree of porosity in the graft material to permit maximum healing is the need to control hemorrhage and prevent undue blood loss at the time flow is re-established through the vascular prosthesis. Of the many different methods which have been tried to solve this problem, those in use at the present time include (1) the use of fuzzy or untwisted yarns to fabricate grafts, (2) the development of tightly knitted materials, (3) the development of extremely tight weaves, (4) heavy calendering of fabrics, (5) preclotting of the graft material with the patient's blood prior to implantation of the graft, (6) sequential opening and closing of the flow through the implanted graft at the time of re-establishment of flow to permit the deposition of fibrin in the interstices, and (7) partial sealing of the pores of the grafts with such nonabsorbable materials as Kel-F, silicone, Silastic, and microscopic fragments of the parent fiber. Extremely tight weaves have been particularly successful in solving the problem of immediate blood loss since these weaves are not only blood tight but also are very nearly air tight. However, the virtual complete loss of porosity in these materials may well lead to the same degree of poor healing as has previously been noted in grafts made of compressed Ivalon sponge.[6]

The use of permanent and nonabsorbable materials to fill the pores of a loosely knitted material simply converts the desirably porous material into a solid tube that would lead to the disadvantages mentioned. These studies have shown perfect healing of the gelatin-coated grafts at the end of 4 months with no evidence of gelatin remaining in the tissues. Though slightly delayed, the healing process is otherwise equal in every respect to that seen in the control grafts untreated with gelatin. The final comparison of gelatin sealing to other methods of treating grafts for blood loss awaits further trial. On the basis of this experimental work, an extensive clinical trial of this method seemed indicated and is underway at Minneapolis General Hospital.

SUMMARY

The problem of preventing excessive blood loss through the pores of knitted vascular prosthetic grafts has been approached in our laboratories with a technique of impregnating the graft with an impervious film of baked gelatin. The baked gelatin film has sharply limited or prevented blood loss from porous knitted grafts without producing any detrimental side effects in the experimental animals. In a carefully controlled series, 138 grafts have been implanted into 69 dogs as controlled pairs. The gelatin-coated grafts have been distinguishable from the untreated grafts only in terms of a 10 per cent delay in the total healing time. The gelatin has been absorbed within 20 days and there have been no untoward side effects such as increased thrombosis in the grafts or increased inflammatory reactions in the tissues about the grafts. A clinical trial on an expanded basis appears necessary now to determine the effectiveness of this method in clinical surgery.

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The silicone-foam diagnostic enema. I. An assessment with surgical specimens of human sigmoid colon

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This presentation is the first in a series which will describe the development of a diagnostic technique designed for the discovery of small abnormalities in the mucosa of the human large intestine. The investigation is particularly concerned with the midsigmoid colon because it is beyond the range of the sigmoidoscope and is frequently difficult to study roentgenographically.

The need for a reliable and thorough method for the examination of the interior of the large intestine can be appreciated after a review of the diverse and inventive approaches which have evolved in the history of colon diagnosis. Radiography of the colon began with the radiopaque enema.[11] This was followed by the double-contrast enema,[8] the use of semitransparent media,[14] the addition of overexposed roentgenographic films,[19] and high-voltage technique.[10] Subsequently, chemicals such as tannic acid[20] and isatin[22] were added to the barium sulfate, and later external bag compression, filling of the urinary bladder,[3] and the Chassard-Lapine[18] and cephalad-oblique-prone projections[7] were introduced. Proctosigmoidoscopy was begun with the introduction of the proctosigmoidoscope. The technique was extended by lengthening the barrel, by coloscopy through colotomies,[6] and by use of the traction sigmoidoscope,[17] the suction-lavage sigmoidoscope,[21] the lucite Y tube,[9] and, most recently, the flexible coloscope.[13] The third avenue of approach has been cytologic study of material obtained by bulb pipetting,[16] sigmoidoscopic aspiration,[23] enema washing,[15] direct tissue smearing,[5] and millipore filtration of colonic washings.[12]

From this array of techniques useful combinations have emerged and are applied today regularly. The reliability of any combination, however, is not absolute, nor is the applicability universal for the discovery of small sessile cancers of the large intestine—especially when they are located in the region of the sigmoid colon. A recent evaluation of the accuracy of cytologic study of material collected above the reach of the sigmoidoscope showed only 4.3 per cent reliability.[4] Even with sigmoidoscopes of advanced design, the sigmoid colons of 25 per cent of patients could not be satisfactorily examined because of anatomic peculiarities.[17] Coloscopy is attended by an appreciable risk of secondary wound infection[2] and is not satisfactory for the examination of the colonic flexures. The accuracy of the barium enema, with all of its modifications, has seldom exceeded 90 per cent; in fact, reliabilities as low as 28 per cent have been reported.[1]

Because of these limitations attempts were made to find a method for making retrievable mucosal molds which have accurate de-

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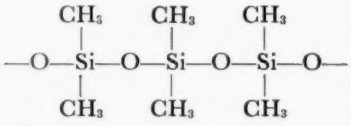
Fig. 1. Semco industrial sealant gun used for injection of silicone foam.

tails of mucosal relief and retain sufficient numbers of mucosal cells to permit accurately located cytologic sampling in the colons of living persons.

The requirements for such a mold are that it be (1) a liquid capable of being converted to an elastic, compressible, readily extrudable solid; (2) non-toxic in both the liquid and solid states; (3) available in quantity, easily handled, and inexpensive; (4) a material (in the liquid phase) with a low viscosity and a high miscibility with radiopaque media; (5) converted from liquid to solid in the bowel rapidly, predictably, and endothermically; and (6) a material (in the solid phase) that is spongy, cohesive, and freely and atraumatically separable from the mucous membrane.

Several sol-gel systems and many plastics were tried and eventually the criteria were met by silicone elastomers. Through the cooperation of the Dow Corning Center for Aid to Medical Research a silicone rubber (Silastic RTV 502*) was selected as the basic component. The molecule of this polymer is a chain of several thousand alternate silicon and oxygen atoms with side branches of short aliphatic radicals (predominately methyl groups):



Polymerization occurs at room temperature and is effected with stannous octoate by a crosslinking of opposing methyl groups at intervals of several hundred monomer units. This reaction converts the liquid prepolymer into a synthetic rubber in from 3 to 10 minutes, depending upon the amount of the octoate added to it. To provide compressibility in the polymer, a silicone rubber foam (Silastic Q-3-0030*) was added to the Silastic RTV 502. This intumescent releases hydrogen gas (0.4 moles per 100 Gm. of Q-3-0030) during polymerization and this gas renders the polymerized mixture spongy. The addition of this hydrogen-evolving silicone does not significantly affect the surface impression detail nor does it impair the cohesiveness of the final product. It does, however, greatly increase the compressibility of the polymer and thereby increases the ease of extrusion. Stannous octoate is also the polymerizing agent for this compound.

The viscosity of a mixture of these two silicones in their prepolymeric phase ranges from 6,000 to 50,000 centistokes, depending upon the proportions of Silastic RTV 502 and Silastic Q-3-0030 added. However, with any combination of these two silicone prepolymers, a relatively viscous fluid is produced. By the addition of a cyclic dimethyl polysiloxane (Silicone Fluid 200, 20 centistokes*) to such mixtures the viscosity may be appreciably reduced. This facilitates giving the fluid as an enema. The character of the polymerized elastomers is not significantly altered if Silicone Fluid 200 represents less than 30 per cent of the prepolymer mixture. Being cyclic, Silicone Fluid 200 does not crosslink and therefore needs no polymerizing agent.

As a final step in the compounding of the

*Dow Corning Corporation, Midland, Mich.

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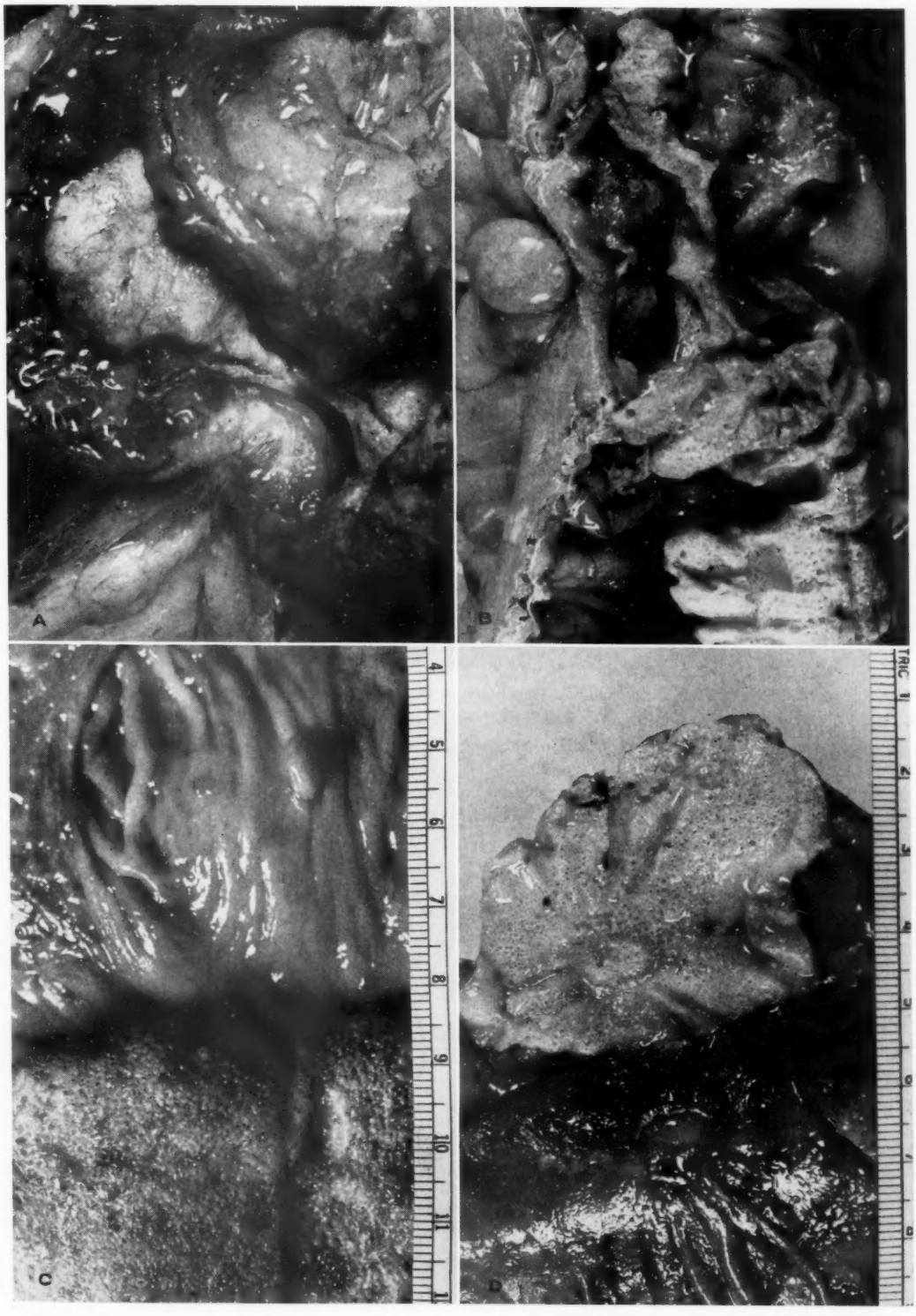

Fig. 2. *A* and *B*, Specimens of constricting adenocarcinoma of the colon with silicone-foam molds within the lumina. *C*, Mucosal polyp of the colon adjacent to silicone-foam impression of the lesion. *D*, Small ulcerating carcinoma of the colon with facsimile of the lesion imprinted on silicone foam.

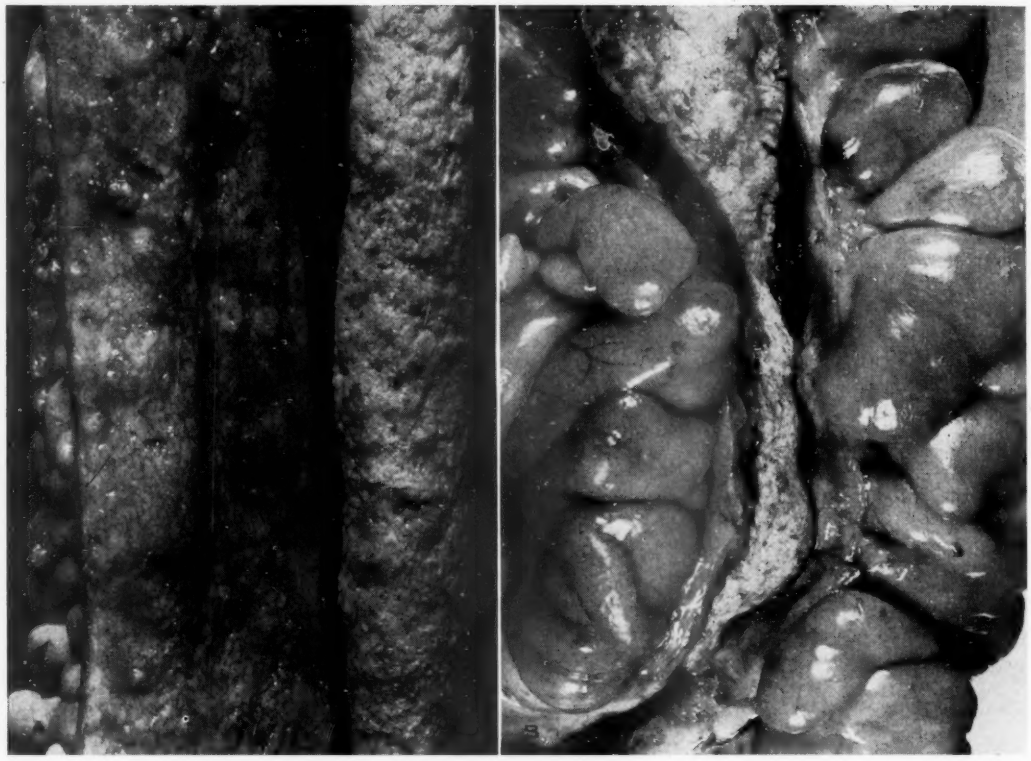

Fig. 3. *A,* Mucosal surface of colon involved with ulcerative colitis (left), and intraluminal silicone-foam mold of same region (right). *B,* Surgical specimen of colon with ulcerative colitis and signet-ring cell carcinoma containing intraluminal silicone-foam mold.

enema, a radiopaque material was added to the silicones so that the inflow could be observed fluoroscopically and the extent of the filling controlled safely and with certainty. Diatrizoate sodium (Hypaque*) was chosen empirically.

Various combinations of the 5 agents were compounded and the mixture finally selected for experimental use was Silastic RTV 502, 50 Gm.; Silastic Q-3-0030, 50 Gm.; Silicone Fluid 200 (20 centistokes), 10 Gm.; diatrizoate sodium, 25 Gm. in 20 ml. water; and stannous octoate catalyst, 2 ml.

First it was introduced into washed pieces of resected human colons. The distal line of resection was opened and through it the bowel was filled with the silicone-foam mixture administered from an industrial sealant gun† (Fig. 1). This apparatus contained the silicone fluid in a disposable polyethylene cartridge and extruded it at a uniform rate by a pressure of 5 pounds air per square inch. The specimens were filled completely but were not distended beyond their natural dimensions. Polymerization required approximately 10 minutes; during this period the bowel was massaged gently to simulate peristalsis.

Upon completion of polymerization, the specimens were carefully opened along the antimesenteric borders. Incisions into the rubber mold and, in specimens with tumors, incisions into the tumor were avoided. Photographs were made with the molds in place and they were then removed from the specimens, suspended in Hartman's solution, agitated, and dried for study of imprint detail. Cellular suspensions were taken to the Cytological Laboratory of Barnes Hospital for centrifugation and microscopic examination.

*Winthrop Laboratories, New York, N. Y.
†Semco Sales and Service, Inc., Inglewood, Calif.

RESULTS

Five surgical specimens of the sigmoid colon, each containing advanced, constricting

adenocarcinomas, were studied (Fig. 2). In one of these specimens an adenomatous polyp was also found—its greatest dimension was less than 1 cm. (Fig. 2, *C*). In another specimen, a second carcinoma attended the major lesion (Fig. 2, *D*). The smaller malignancy remained occult until the silicone mold was studied. Upon recognition of it, the mold was transected so that the silicone reproduction of both the major and minor lesions could be submitted separately for cytologic study.

Two colons involved with advanced ulcerative colitis were examined (Fig. 3). The second specimen contained a signet-ring cell carcinoma in addition to colitis.

The cytologic examination of the cellular debris in every malignant lesion gave positive indications of carcinomatous cells (Fig. 4).

An even better appreciation of the precision with which the silicone-foam mixture records surface details can be obtained by observing a mold of the human palm (Fig. 5). Here the relief is more intricate and also more familiar than the mucosal surface of the large intestine.

DISCUSSION

It is evident from a study of these molds that silicone elastomers, as combined here, record minute variations on any surface they contact and consequently give information that is more discrete and more precise than that yielded by radiographic methods.

Silicone elastomers are produced commercially and are readily available. Their cost, when used in the quantities described in this study, does not exceed 1 dollar per examination. With the accessory equipment used by us, the bowel is easily filled.

The physiologic inertness of silicone compounds has been their most attractive characteristic for medical application. Toxicity data are presented in a subsequent report.

The credibility of any technique based on direct removal of malignant cells from an occult region is statistically suspect. For this reason, the 100 per cent recovery of malignant cells recorded in this report is not meant to predict a similar recovery rate clinically. The mold's excursion may denude it of some, or perhaps all, of the cells attached at the site of impression. A clinical trial will expose this difference.

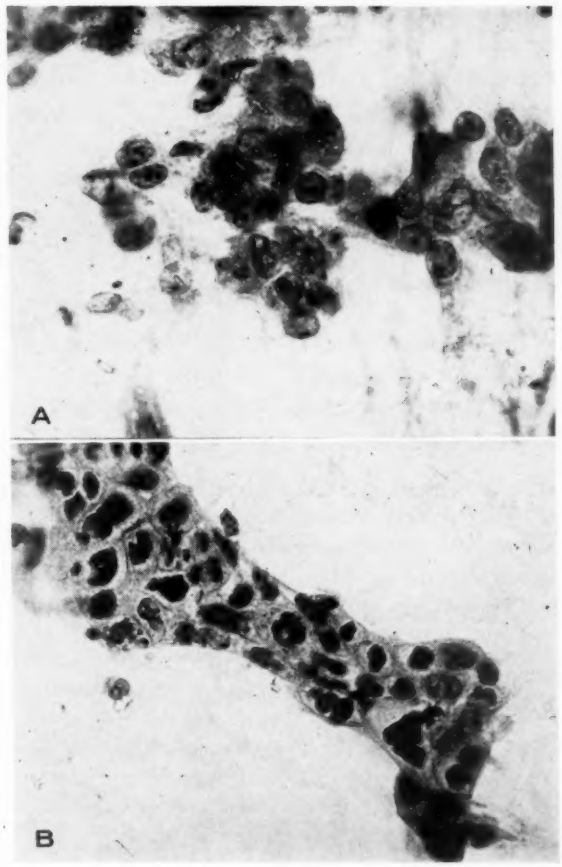

Fig. 4. *A* and *B*, Malignant cells obtained from Hartman's solution suspension of silicone-foam molds.

Fig. 5. Photograph of a silicone-foam impression of the human hand to demonstrate the fine detail which the material records.

SUMMARY

A newly conceived technique for the diagnosis of disease in the sigmoid colon is introduced. The method is based on the proposition that a retrievable foam rubber intraluminal mold of the distal intestine would be useful in an assessment of the character of its mucosal surface. A mixture of silicone elastomers which can be converted at body temperature from a liquid prepolymer to a foamed polymer is presented. The results in reproducing surface abnormalities and recovering malignant cells from human surgical specimens are recorded.

The authors wish to thank Dr. William F. Newton for his support in this study.

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The silicone-foam diagnostic enema. II. An experimental evaluation in dogs

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Polysiloxane elastomers, when polymerized within segments of resected intestine, produce synthetic foam rubber molds which take true intraluminal impressions of the bowel mucosa. These elastomers have a sufficiently low viscosity to permit easy injection. They polymerize at room temperature and provide a precise and permanent record of the mucosal detail.[1]

Before such materials can be used to examine the colon of living men, it must be shown that (1) the injection of the prepolymer can be effected safely, efficiently, and painlessly, (2) the material does not injure the intestine in any way, and (3) the expulsion of the polymerized mold can proceed spontaneously, rapidly, completely, and without pain even from a bowel with polyps, diverticula, strictures, or many loops. To assess these criteria we have used the silicone-foam enema in the colons of dogs.

MATERIALS AND METHODS

The silicone-foam enema used in these experiments is composed of Silastic RTV 502,* 20 Gm.; Silastic Q-3-0030,* 50 Gm.; Silicone fluid 200 (20 centistrokes),* 30 Gm.; diatrizoate sodium,† 25 Gm. in 20 ml. of water; and stannous octoate* 2 ml.

This mixture is somewhat different than the one previously reported.[1] It is less viscous as a prepolymer and softer as a polymer, but retains the other important characteristics of the original mixture.

The preparation of anatomic deformities in the colon. In order to make the straight colon of the dog resemble the human sigmoid colon, loops were made in it by sewing the serosa to form consecutive C curves. Sequences of 2 to 4 loops were made. The most caudal one was placed within 15 cm. of the anus in order to simulate the position and shape of the human sigmoid.

"Polyps" were made by incising the surface of the colon 10 cm. from the anus and preparing a 2 by 2 cm. pedicle. The pedicle was made into a serosa-lined tube and suspended in the intestinal lumen as the bowel wall was closed over it.

Diverticula were prepared by excising circles of serosa and circular muscle of 1 cm. diameter from the lateral portions of the antimesenteric surface of the colon in a zone 10 to 20 cm. from the anus. The underlying mucosa was everted through these defects and the muscular margins were secured to the intact peripheral serosa.

Strictures were produced by placing bands of vinylite, 1 cm. in width, circumferentially about the bowel and securing the resultant ring to the serosal surface with silk sutures. One to 2 weeks later these bands were removed and a partially obstructing stricture remained.

Evaluation of the physical characteristics of the enema. During polymerization the

The latter phase of this study was supported in part by a University Institutional Grant from the American Cancer Society.

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†Winthrop Laboratories, New York, N. Y.

silicone mixture expands. This of course could be dangerous. Consequently, we measured this volume change by placing the prepolymerized material in an open cylinder, measuring the height of the column, effecting polymerization, and then again measuring the column at the completion of the reaction.

The tendency for the material to push itself through small channels during polymerization was ascertained with Penrose drains (4 cm. wide) placed through a series of holes of varying diameters drilled through a rubber block 2 cm. thick. This device was designed on the premise that a human colonic tumor which had grown to completely encircle the bowel would have a longitudinal dimension of no less than 2 cm.[7] The proximal half of the drain was filled with 50 Gm. of silicone enema and sealed. Polymerization was allowed to go to completion and the weights of the polymerized rubber on each side of the constriction were compared.

One component of the enema, Silastic Q-3-0030, imparts the spongy character to the polymer by releasing hydrogen gas during the polymerization. Although much of this gas is held in the polymerized elastomer, some of it escapes. Because of the explosiveness of an oxygen-hydrogen mixture, the amount of hydrogen escaping from the polymer was measured by placing 50 Gm. of the enema mixture in a sealed condom and polymerizing it. The volume of hydrogen gas evolved from the polymer was measured by Archimedes' principle. The possible explosiveness of this gas in vivo was further appraised by giving a silicone-foam enema to an anesthetized mouse which had an empty colon. During polymerization an open flame was held to the anus. This flame was touched to the portion of the enema that extruded from the rectum as the prepolymers expanded. After polymerization, the animal was killed and the flame was held close to the intestinal loops as they were opened with scissors to allow the egress of any gases retained in them.

A deformation analysis of the polymerized elastomer was made. Tangential stress was applied with an infant's sphygomomanometer wrapped about a column of the foam 4 cm. in diameter. The strain was recorded on a centimeter scale by measuring the deflection of a pointer, the fulcrum of which rested at the edge of the pressure cuff with its proximal tip at the center of the compressed area. Longitudinal stress was measured by applying weights to the same foam column and recording strain as the reduction in height per unit added weight to an 8 cm. column of polymer. The longitudinal measurement was made with the foam column held in a lubricated polyethylene tube and also with the foam surrounded only by air.

The rise of temperature of the mixture during polymerization was measured with initial temperatures of 25, 30, and 35° C.

The tissue reaction to silicone foam was investigated by isolating a segment of dogs' colon in vivo and filling it with the enema mixture. One week later this intestine was removed and examined. Multiple biopsies were also made of the mucosa of the distal colon through a sigmoidoscope at various

A *B*

Fig. 1. *A,* Roentgenograph of radiopaque silicone-foam enema within the colon of a dog prepared with 2 C loops. *B,* The same silicone-foam mold after expulsion shows the configurational stability of the material. It has returned to the position it held in the bowel.

intervals after exposure to the silicone-foam enema. A final study was made by injecting 60 Gm. of the enema directly into the peritoneal cavity and studying the tissue response 1 week later.

Evaluation of the injection of the enema. Initially the enema was released into the rectum through the nozzle of the sealant gun.[1] Later the nozzle was attached to a Foley catheter fitted with a 30 c.c. Bardex bag.*

Early in this study all animals were anesthetized during the injection, but as confidence was gained this was found unwarranted and thereafter the only pre-examination preparation was the giving of a 4.5 fluid ounce sodium phosphate/sodium biphosphate Fleet† enema.

Cineradiography was used to observe the rate and character of the injection in 3 instances. A head of pressure of 5 pounds per square inch of compressed air was used in these and all subsequent studies for expelling the prepolymerized enema from the sealant gun into the rectum.

Evaluation of the expulsion of the enema. Immediately after polymerization of the silicone foam (determined by observation of the material which remained in the sealant gun cartridge) the dogs were placed on a radiographic table and a right posterior-oblique scout film of the abdomen was made. The animals were then allowed to move about the room and pass the cast.

RESULTS

Anatomic deformities. A silicone-foam enema was administered to a dog with the colon in the natural state. The examination was then made in dogs prepared with redundant loops (Fig. 1), a polyp (Fig. 2, *A*), diverticula (Fig. 2, *B*), and strictures (Fig. 3). The severest stricture produced had a diameter 35 per cent as great as the diameter of the uninvolved bowel cephalad to the constriction.

*Hamilton-Schmidt Surgical Co., St. Louis, Mo.

†C. P. Fleet Co., Inc., Lynchburg, Va.

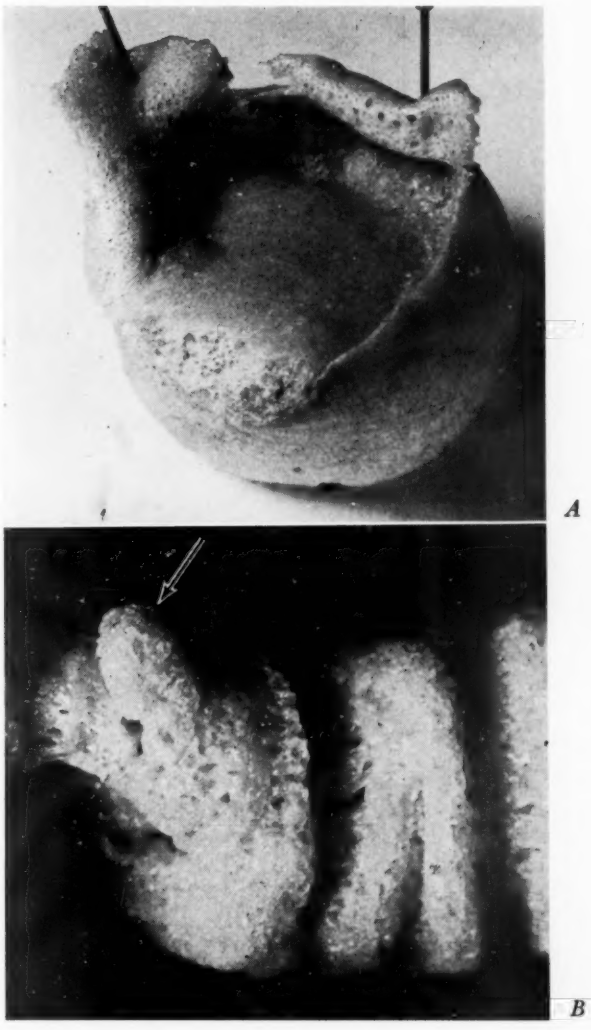

Fig. 2. *A*, Photograph of "pocket" in silicone-foam enema produced by experimentally prepared "polyp." This view is from the base of the "polyp" and is directed caudally. *B*, Silicone-foam impression of surgically prepared diverticulum *(arrow)*.

In every instance the mold was expelled promptly and without assistance; there were no manifestations of pain. Not all specimens, however, were passed as single units—the polyp mold and several of the stricture molds were expelled in 2 parts with the second portion following the first 4 to 8 hours later. In each case in which a fracture appeared it developed at the narrowest part of the mold. Repeat examinations in the same animals were not always attended by fracture of the mold.

Examination of the molds clearly showed

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Fig. 3. Photograph of silicone-foam mold lying on a roentgenograph of the same mold before expulsion. The diameter of the constricted zone *(arrow)* is less than one-third that of the greatest diameter above it.

the abnormalities which had been prepared in the colons. The specimen of the 4-loop bowel provided an especially convincing demonstration of the ability of the foam to retain the geometrical configuration that was present at the time of polymerization.

Physical characteristics of silicone foam. The silicone-foam enema had a viscosity of 2,000 centistokes at the time of injection.[6] During polymerization the volume increased 140 per cent and the temperature rose less than 3° C. (maximum temperture rise 2.3° C.).

During polymerization, 70 cc. of free gas escaped the mold. When it was ignited in vitro a flame 5 cm. in height rose from it and burned for approximately 2 seconds; an explosion did not occur. When the flame was applied to the anus or over the incised, distended bowel of a mouse, neither an ignition nor explosion resulted.

The overflow of foam through a constriction was found to be a function of the size of the constriction being directly related to its cross-sectional area:

$$\frac{\text{cross-sectional area of constriction}}{\text{cross-sectional area of proximal lumen}} \times 100$$

compared with

$$\frac{\text{weight of silicone foam above constriction}}{\text{total weight of silicone foam administered}} \times 100$$

37.5%:39.5%
50 %:42.5%
62.5%:45.7%

The deformation analysis (Fig. 4) showed that the strength of the normal bowel (> 200 mm. Hg^2) would be capable of compressing the foamed elastomer to a cross-sectional area less than 50 per cent of its prestressed size.

A tissue response attributable to the silicone was not found. The peritoneal cavity was involved with a moderate inflammatory reaction which was interpreted as an effect of bacterial contamination in view of the complete absence of response in all other material examined. The wall of the colonic pouch which had been in contact with silicone foam for 1 week, as well as the immediate and delayed rectal biopsies, showed no reaction (Fig. 5).

Injection and expulsion of the enema. Administration of the silicone foam through a rectal catheter identical to that used for barium enema examinations was functionally satisfactory. However, because of the relative viscosity of the prepolymer, it was found necessary to continue to employ the sealant gun instead of the traditional gravity flow technique used for barium enemas. The animals showed no signs of pain during the injection or during the expansion of the material as it polymerized.

In these studies, as in all examinations of the colon, mucosal cleanliness is essential. Without exception, the Fleet enema prepared the lower colon so that a satisfactory examination could be performed.

Cineradiographic observations of the injection showed a steady, controlled inflow

of the silicone foam. The Hypaque concentration was satisfactory for good visualization at all times.

Expulsion of the molds began 1 to 5 minutes after the animals were allowed to stand. It was complete 3 to 10 seconds later. There were no manifestations of pain.

DISCUSSION

Our attempted simulations of human polyps, diverticula, strictures, and redundant loops in the colons of dogs are admittedly crude. However, they did serve to demonstrate that the silicone foam is capable of accurately recording the forms. In addition, they demonstrated that the silicone mold is sufficiently pliable to prevent injury to any part of the bowel during extrusion. We believe that it can be safely introduced into man and will provide a very accurate and permanent record of the structural details of the colon of living persons. The value of such records is obvious.

Although we could not demonstrate that the hydrogen evolved during polymerization would burn or produce an explosive mixture in vivo, we still believe that this constitutes a potential hazard and consequently warrants the taking of care to avoid flame or sparks. All fluoroscopic and electrical radiographic equipment must be well insulated.

That no mucosal injury occurred during the silicone-foam enema was expected because the exposure of the gastrointestinal tract to other polysiloxanes for long periods produced none.[3, 4] The short time that the silicone-foam enema remains in the colon is insignificant compared to the lengths of time that silicone elastomers have been left subcutaneously, intramuscularly, and intraperitoneally without producing discernible changes in the tissues contacting them.[5]

The use of a Foley catheter to introduce the enema into the rectum serves to protect the area from the rigid polyethylene nozzle of the sealant gun and prevents overfilling the rectal ampulla with the polymer. However, this method of introducing the silicones is cumbersome and expensive and therefore we are now attempting to overcome these problems with a flexible, disposable apparatus.

Our experience with the silicone-foam enema in human beings is in its early stage. In 50 instances of its employment, no pain has been experienced during the introduction or polymerization of the material. The elastic casts have recorded the details of the sigmoid colons of living human beings as accurately as those of the dogs' colons.

SUMMARY

The application of a new technique in which silicone elastomers are used for recording mucosal detail and gross anatomic configuration of the sigmoid colon of dogs

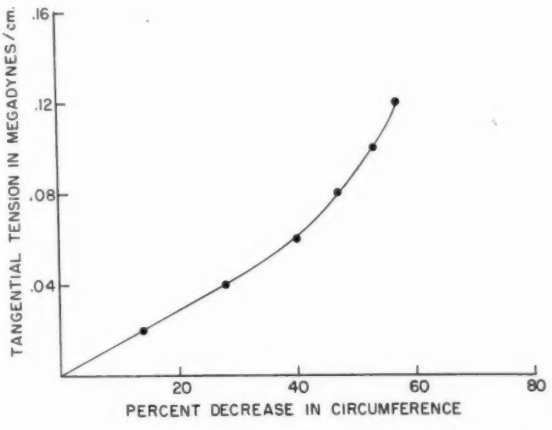

Fig. 4. Graph which demonstrates the compressibility (tension-circumference relationship) of the silicone-foam enema after polymerization.

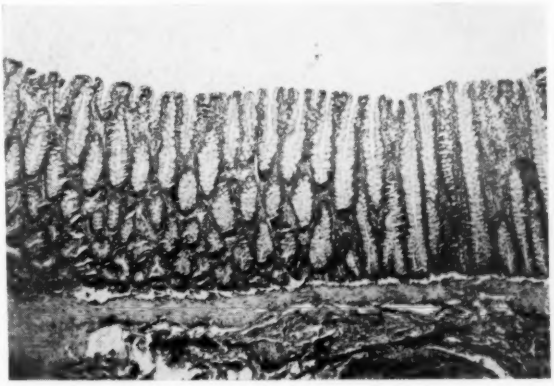

Fig. 5. Photomicrograph of intestinal mucosa exposed to the silicone-foam enema for 1 week in an isolated bowel segment.

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in vivo is presented. The physical properties of the material and the conditions of its administration, polymerization, and evacuation have been investigated. An absence of tissue response has been encountered. Human studies, to date, closely parallel the experiences with dogs.

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The healing wound in experimental diabetes

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Patients with diabetes are subject to some diseases for which they must undergo operation more frequently than the population at large, but as clinical knowledge and surgical techniques improve, diabetes is becoming less of a barrier to elective surgical procedures. Because the aging of the population brings with it an increase in the incidence of diabetes, a proper understanding of the effect of diabetes on surgical procedures and wound physiology assumes a greater importance than ever before.

Statements regarding the relationship between diabetes and wound healing can be found frequently in the literature. Eagleton,[2] writing in 1904 before the introduction of insulin, observed that even in the absence of infection diabetic wounds were slow to granulate and to heal. More recently it has been stated that there is no retardation of healing if therapy is adequate, and that diabetes has no effect on wound healing in the absence of infection.[9] Unfortunately, there are very few reports of a well-controlled clinical or experimental investigation to substantiate these opinions. Bennet,[1] of the Mayo Foundation, was one of the few to look into this problem critically. He examined standard abdominal incisions in dogs after total pancreatectomy and without insulin supplementation, using normal dogs as controls. He found that healing proceeded in an abnormal fashion in the diabetic dogs. The tissues were edematous, fibrin deposits were delayed and decreased in amount, the cellular reaction was excessive, blood vessel formation was slow, and thromboses were increased. Although he answered many questions about the method of healing in diabetes, he left unanswered the essential point—do diabetic patients heal as rapidly and as completely as normal persons, and if not, does control with insulin influence the result? The present study is an attempt to provide some additional information on this subject.

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METHODS AND RESULTS

White male albino Wistar rats were used. Diabetes was induced by subcutaneous or intraperitoneal injection of alloxan, 150 mg. per kilogram, in rats which had been fasting for 48 hours. Each animal had received 0.25 U. of regular insulin 1 hour prior to the alloxan administration. Diabetic rats were given 1.5 U. of protamine zinc insulin (PZI) subcutaneously daily to prevent cachexia and dehydration. Blood sugar levels in this group remained over 300 mg. per cent. A second group was made diabetic in a similar manner, but after the diabetes was well established they were given 7 to 9 U. of PZI daily. This was sufficient to maintain the blood sugar levels in the normal range of 65 to 135 mg. per cent. This group is referred to as insulin controls. A third group of normal rats of the same colony which never received alloxan was also used.

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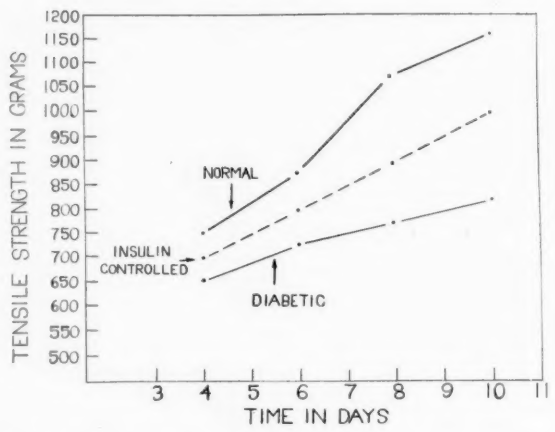

Fig. 1. Gain in tensile strength of sutured incised wounds.

Incised wounds. Rats which weighed between 200 and 250 grams were anesthetized intraperitoneally with pentobarbital. Under sterile conditions, the skin and subcutaneous tissue of the upper abdomen were incised in the midline. A 4 cm. vertical incision through the musculofascial layer, including peritoneum, was then made in the left upper quadrant of the abdomen. This incision was then repaired with 7 interrupted simple sutures of No. 4-0 silk. These sutures were placed exactly 0.5 cm. apart and 0.5 cm. from the ends of the incision. Each suture took in the entire musculofascial wall, including peritoneum but excluding subcutaneous tissue and skin. Each suture was tied with a square knot and another squared throw, and the ends cut 1 mm. from the knot. The skin was then closed with a continuous silk suture.

The surviving rats were sacrificed on the fourth, sixth, eighth, and tenth postoperative days. The abdominal skin was removed and the remainder of the abdominal wall excised. Three strips were cut from the wound, each strip 1 cm. wide and containing 2 of the previously placed sutures. All 3 strips were tested for tensile strength on a motor-driven tensiometer, calibrated to read the breaking force to the nearest 5 Gm. Most, but not all, of the specimens broke at the suture line. The results are summarized in Table I and are plotted graphically in Fig. 1. Parabolas were constructed for each of the 3 groups and the algebraic equivalents determined. An F test on the residual variation of the parabolas indicated that the 3 parabolas differed significantly from each other ($p < 0.001$) and that this difference lies in the level rather than the slope of the curve.

Open wounds. Rats weighing between 160 and 210 grams were used. Under intraperitoneal pentobarbital anesthesia the right flank was shaved and a square full-thickness skin defect, 2 by 2 cm., created. Sterility was not observed. Hemostasis was established and the wound left undressed. The wound was inspected daily until full healing occurred. The absence of any visible granulation tissue was used as the end point of healing.

Again, 3 separate groups of animals were studied—diabetic rats, insulin controls, and normal rats. The results are presented in Table II. There is no significant difference between any 2 of the 3 groups, despite the fact that the diabetes was so severe that the diabetic rats gained an average of only 1.5 grams per day compared to 4.7 grams in the normal group and 3.7 grams in the insulin control group. Wound infections were most common in the diabetic group, but could be noted to some degree in all groups. Fungus

Table I. *Tensile strength of sutured incised wounds*

Day of sacrifice	*Study group*	*No. of animals*	*Average tensile strength of wounds (Gm.)*	*Standard deviation*
4	Normal	11	741	73
4	Insulin control	11	700	64
4	Diabetic	15	645	77
6	Normal	11	873	73
6	Insulin control	11	794	93
6	Diabetic	10	732	52
8	Normal	11	1,088	69
8	Insulin control	10	900	131
8	Diabetic	11	789	101
10	Normal	11	1,161	83
10	Insulin control	10	1,003	126
10	Diabetic	11	884	112

Table II. *Healing time of open granulating wounds*

Study group	No. of animals	Average weight gain (Gm./day)	Range of healing time (days)	Average healing time (days)	Standard deviation
Normal	27	4.4	12-20	15.9	2.3
Insulin control	19	3.5	13-28	17.1	3.9
Diabetic	29	1.5	13-21	15.8	2.2

infection occurred in 3 of the diabetic animals but in none of the others.

DISCUSSION

Diabetes alters the healing of wounds in a number of ways, not all of which are simulated by alloxan injection in the experimental animals. The main metabolic difference between alloxan diabetes and clinical diabetes is in the relatively frequent occurrence of ketosis in the latter condition. There are some microscopic differences also. The pancreatic beta cells are absent or markedly reduced in number in the alloxan form, while in the clinical type the decrease in the number of beta cells is not as marked, although with special stains, 60 per cent of diabetics can be shown to have a partial or complete degranulation of the cells.

The similarities between the 2 forms, however, are greater than the dissimilarities. Hyperglycemia, glycosuria, insulin deficiency, and hyperlipemia are common to both. Both are aggravated by thyroxin and ACTH, while both are ameliorated by adrenalectomy, fasting, high-fat diet and insulin. Some authors even believe that alloxan plays a part in clinical diabetes. Loubatieres[7] was able to isolate alloxan and its metabolites in greater amount from the blood of diabetic patients than from nondiabetic control subjects. The vascular changes which are associated with well-established diabetes probably did not occur in our experimental animals in which the diabetes was of short duration. In all other important respects, the experimental situation is probably comparable to the clinical.

It is pertinent that all 3 curves in Fig. 1 have the same slope. In other words, the rate of healing from the fourth to the tenth day does not differ significantly from group to group. This is confirmed in the second part of the study, in which rate (the time factor) was the only variant investigated. Experimentally, almost all tissues studied (cornea,[4] skin, fascia, muscle, stomach, and so forth[6]) have the same rate of healing. It is not surprising that the same tissues from diabetic and nondiabetic animals of the same species should also heal at the same rate. Nevertheless, the curves in Fig. 1 do differ significantly from one another in height. The amount, or strength, of healing differs; for example, a healed corneal wound is never as strong as a healed skin or fascial wound.

The mechanisms by which wounds heal are still only imperfectly understood, and the reasons why they heal are even more conjectural. It is not surprising, therefore, that we have few hints as to the reason for the difference in healing between diabetic and nondiabetic patients. Bennet implied that this difference was due, in part, to the differences in sugar content of the local tissues. He quoted Trimble and Carey,[8] who found that the true sugar content of skin and muscle was roughly proportional to the blood sugar level. The skin and muscle sugar levels in diabetic patients were approximately twice those of nondiabetic patients. Greene and associates[5] analyzed the infection rate and rate of healing of the wounds of 324 diabetic patients, and related these rates to the strictness of control of the diabetes as evidenced by the blood sugar levels. They could find no apparent relationship between the blood sugar level and wound infections or delayed wound healing. Forscher and Cecil[3] have shown that tissue levels of tyrosine, desoxyribonucleic acid, and acid-soluble organic phosphorus increased in the wounds of normal rats but failed to increase in the wounds of diabetic rats, while the rate of increase

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in tissue glycogen was greater in the wounds of diabetic rats. They implied that altered wound healing in diabetic patients was due to these differences from the normal. In our study the group of insulin controls had normal blood sugar levels but had altered healing, although the healing was more nearly normal than in the rats with uncontrolled diabetes.

SUMMARY AND CONCLUSIONS

1. The healing of sutured incised wounds and open granulating wounds has been studied in rats with alloxan diabetes.

2. The healing of sutured incised wounds is impaired in severely diabetic animals; this impairment is partially ameliorated by strict control of the diabetic state.

3. The healing of open granulating wounds appears to proceed at a normal rate in rats with well-controlled and poorly-controlled diabetes.

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Paradoxical variation of the femoral pulse in occlusion of the iliac artery

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Presence or absence of the femoral pulse is an important guide to the evaluation of a patient suspected of having arterial occlusive disease of the lower extremities. A weak or absent femoral pulse denotes a proximal occlusive lesion, and if a reconstructive operation is indicated the precise location of the lesion may be verified by aortography. A strong femoral pulse is often interpreted as an indication that no significant proximal lesion exists. Routine aortography in patients with good femoral pulses, however, will demonstrate that many do have proximal lesions of the aorta or iliac arteries. Although these arteries may not be completely obstructed, an appreciable number will be found to have a marked stenosis of a degree greater than might be expected from the good character of the femoral pulse. In this situation, surgical relief of a distal occlusive lesion in the leg may fail immediately because of inadequate proximal blood flow or at a later date because of progression of the proximal lesion with reocclusion of the distal operative area.

The occurrence of a good femoral pulse below a significant occlusion in the absence of distal obstructive lesions is seen only rarely, usually in patients in the younger age group. In these patients, large collateral channels can be demonstrated by physical examination or arteriography.

The association of proximal iliac lesions with occlusive lesions of the femoral artery has been seen occasionally with good femoral pulses and has raised the question as to what effect multiple occlusive lesions have on the intervening pulse. A series of experiments was carried out in animals with the production of simultaneous iliac and femoral obstructions in an attempt to answer this question.

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METHOD

Four mongrel dogs, which varied in weight from 15 to 20 kilograms were subjected to laparotomy under intravenous pentobarbital anesthesia. The aortic trifurcation and the entire iliac artery on one side were exposed and the iliac artery mobilized from the aorta to the inguinal ligament. All branches except for the pudendoepigastric trunk were ligated and divided. This left a system which simulated the human external iliac artery and common femoral artery with its bifurcation into superficial and profunda branches. The iliac artery in these dogs averaged 4 mm. in diameter at its upper portion and 3 mm. in diameter below the pudendoepigastric branch. The latter averaged 2.5 mm. in diameter. A No. 20 needle was inserted into the midportion of the iliac artery and this was connected by polyethylene tubing to a Statham transducer. Pressures were transcribed on a Sanborn 350 recorder. Base line or control pressures were then recorded and a screw clamp applied gradually to the iliac artery at its origin from the aorta. Sufficient progressive constriction was applied to convert the pulsatile pressure distal to the clamp to a flat, dampened pressure but not enough to produce complete occlusion.

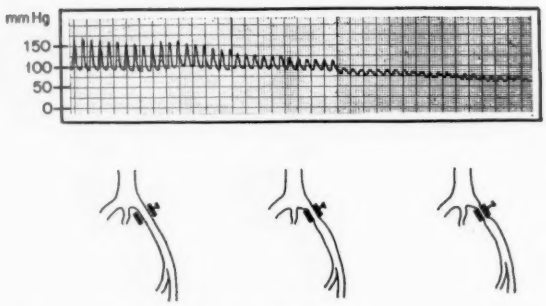

Fig. 1. Application of progressive occlusion to the proximal portion of the iliac artery until dampening of the distal pressure tracing occurs.

In the first portion of each experiment a vascular clamp was then applied to the distal iliac artery to produce complete obstruction. This clamp was alternately removed and applied at intervals of 10 seconds to 2 minutes as pressures were recorded in the artery just above.

In the second stage of the experiment, a bulldog clamp was applied to the distal iliac artery beyond the pudendoepigastric trunk and this was followed by the application of another occlusive bulldog clamp to the pudendoepigastric vessel.

In the third stage of the experiment, a screw clamp was also applied to the distal iliac artery and progressive occlusion produced, then released. Pressures were measured continuously as described.

During each of the portions of the experiment in the 4 animals the proximal occluding clamp was not touched and the upper block remained the same.

RESULTS

The findings in all the animals were similar and are illustrated in representative tracings. The initial, or control, blood pressure was first recorded. The pressure was then noted during progressive proximal occlusion until a pulse pressure well below 15 mm. Hg was obtained (Fig. 1).

Fig. 2 illustrates the first portion of the experiment in which the distal occluding clamp was applied for 10-second periods and released. Application of the distal occluding clamp uniformly resulted in the return of definite pulsatile pressure in the segment above. This pulse pressure was equal to that obtained in the unobstructed artery at the start of the experiment.

Fig. 3 demonstrates the findings in the second portion of the experiment. With the application of the bulldog clamp to the femoral artery the pressure became pulsatile with a pulse pressure averaging 69 per cent of the control value. With the application of an additional bulldog clamp to the pudendoepigastric branch, the pressure rose to normal control levels.

Fig. 4 illustrates the findings in the third portion of the experiment. With the application of the screw clamp distally, the pulsatile pressure gradually returned in the segment above, reaching a peak with complete occlusion of the vessel. As the clamp was again opened the proximal pressure fell and pulse pressure returned to 5 to 10 mm. Hg.

DISCUSSION

These experiments indicated that the dampening effect on the pressure produced by a proximal iliac stenosis could be negated by the production of a distal occlusion. Complete distal occlusion at a level corresponding to the femoral bifurcation uniformly restored proximal pulsatile pressure in the common femoral artery to the control level. The presence of 1 main collateral leaving the obstructed segment was still associated with the return of a pulsatile pressure (Fig. 3), although this pressure amounted to roughly two-thirds of the control pressure. This would correspond to the clinical sit-

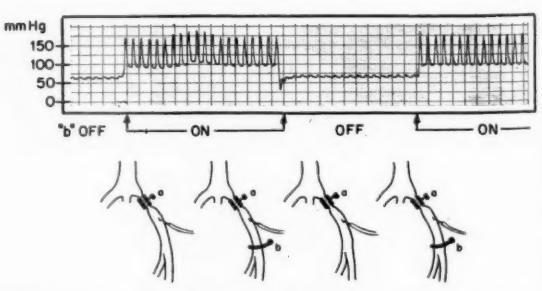

Fig. 2. Dampened pressure tracing produced by the proximal stenosis, relieved by the application of a distal occluding bulldog clamp.

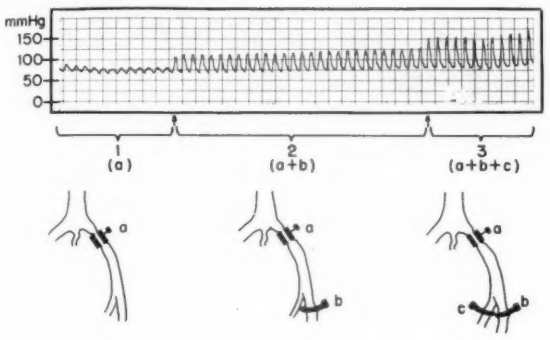

Fig. 3. Dampened pressure tracing produced by the proximal stenosis partially relieved by the application of a bulldog clamp to the superficial femoral artery, then completely relieved by the application of another bulldog clamp to the pudendoepigastric (profunda) branch.

uation in which proximal iliac stenosis existed with superficial femoral occlusion and the profunda femoris represented the only open vessel. Because the quantitation of a palpable pulse is difficult, the pulse pressure was taken as the alternative measurement. In these animals it was noted that a pulse pressure in excess of 20 mm. Hg was associated with a good palpable pulse, and a pulse pressure under 15 mm. Hg with a weak or absent pulse. Thus, complete distal occlusion always resulted in the return of the proximal pulse to the control levels. The degree of occlusion required to restore the pulse was not quantitated, but a collateral branch half the size of the proximal partially occluded artery still permitted the return of the pulse upon occlusion of the distal main vessel.

The explanation for this apparent paradox can be found by the application of Pascal's law, which states that pressure exerted anywhere on a confined liquid is transmitted undiminished to every portion of the interior of the containing vessel. For fluid in motion this law may well not apply, but when, as in the first experiment (Fig. 2), a distal occluding clamp is applied, the segment of artery above is converted into a closed system communicating with the pulsatile pressure in the aorta through the small opening of the proximal occlusion. There is an analogy between the transmission of a pulsatile pressure through the small catheter into the transducer which was used to record pressures in this experiment and the similar transmission of pulsatile pressure through a proximal stenosis into a distally occluded segment of vessel. This simulates a profound obstructive state which is not seen clinically without thrombosis in the segment of vessel in which stasis of blood occurs.

With the use of the screw clamps in this experiment, precise calibration of the size of the block was not possible, although the number of turns required to open the clamp after complete occlusion could be used as a rough guide. Precise determinations of the relative size of the occlusions required to do this are now underway and will be reported at a later date. However, it was our impression in the third experiment (Fig. 4) that even though the distal occluding clamp was gradually opened, the pressure in the vessel just above remained pulsatile until the distal opening was larger than the proximal opening. Thus, it is postulated that when the opening in the distal block becomes larger than that in the proximal block, the pressure in the segment above will rapidly drop off until, with further distal release of the occlusion, the flat pulse tracing secondary to proximal block again occurs.

The results of these experiments, particularly that illustrated in Fig. 3, which comes closest to simulating the clinical situation,

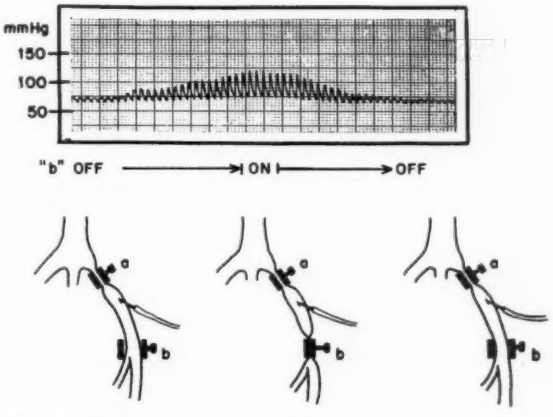

Fig. 4. Dampened pressure tracing produced by the proximal stenosis, relieved by the progressive application of a screw clamp. Gradual release of clamp results in return of dampened pressure.

show that under certain circumstances distal occlusive lesions may augment or restore the pulse which otherwise would be dampened by a proximal occluding lesion. The clinical state in which a weak or absent femoral pulse due to an iliac stenosis may be augmented to a moderate or strong pulse by extensive distal femoral occlusive disease may exist much oftener than has been suspected. This is not to decry the value of the femoral or any other pulse in the assessment of arterial occlusive disease, but to point out that in certain instances, particularly those in which there is associated distal occlusive disease, the pulse may be deceiving. Thus, the presence of a good femoral pulse does not in itself rule out significant proximal stenosis. Complete arteriography is required to evaluate the proximal aortoiliac tree properly when significant femoral occlusive disease exists.

SUMMARY AND CONCLUSIONS

The concept that strong femoral pulses rule out significant proximal arterial occlusive disease is questioned. In experiments performed on dogs, stenotic lesions were produced in the iliac arteries which were sufficient to obliterate the distal pulse. The restoration of a normal pulse in the femoral area was accomplished by the production of a complete distal obstruction. In addition, clamping of the superficial femoral artery in the dog was also sufficient to restore a good pulse in the common femoral artery even though the profunda femoris remained patent.

This suggests that a palpable femoral pulse in association with severe distal occlusive disease of the superficial femoral artery and profunda femoris in man does not necessarily signify lack of proximal disease. Aortography would be indicated routinely in these patients prior to the performance of reconstructive procedures in the legs because the distal operation may fail immediately or be compromised soon thereafter by an unsuspected aortic or iliac occlusive lesion.

Effect of removal of adventitia from arteries: an experimental study

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During the course of experiments concerned with alteration of arterial structure, it became necessary for us to know what changes, if any, could be attributed to the surgical removal of adventitia from the wall of an artery. Little information was available on this subject, and for that reason it seemed desirable for us to record our observations. Knowledge concerning the reaction of an artery to removal of its adventitia is of interest not only to investigators who manipulate blood vessels experimentally, but also to surgeons who perform operations in which arteries are stripped, such as radical operation for cancer, or in which arteries are removed from their usual bed, such as procedures to augment the coronary circulation of the heart.[3]

There is general agreement that the metabolic needs of the walls of large arteries are supplied both by means of the vasa vasorum, which are present in the adventitia and send branches into the media, and by diffusion from the blood within the lumen of the artery.[1, 2, 5] The effect on the arterial wall of destruction of the adventitial source of nutriment has been the subject of a number of investigations. In 1871, Durante mechanically removed as much of the adventitia as he could from the carotid arteries of rabbits and subsequently studied those vessels histologically. He concluded that, with the adventitial blood supply removed, the arteries disintegrated despite continuous flow of blood through them. His investigations were complicated by the occurrence of infection and mural thrombosis.

Lange removed as much of the adventitia as possible from 1.5 cm. lengths of aorta and from carotid and renal arteries in rabbits, and then he surrounded the vessels with a sheath of lanolin and beeswax. Thickening of the intima within the sheathed part of the vessel occurred in less than 12 days. In the course of performing periarterial sympathectomies, Haberland removed as much adventitia as possible from unspecified lengths of major arteries in dogs and rabbits. No damage attributable to disturbances of nutrition in the walls of the vessels was apparent on microscopic examination after postoperative periods of as long as 51 days. Schilling observed intimal proliferation after removal of adventitia from arteries in rabbits, but only at sites where the media had been traumatized. In other studies in which the adventitia was cauterized by heat[19-22] or treated with caustic chemicals,[9, 10, 24] abnormalities of both media and intima were produced.

Removal of adventitia from an artery not

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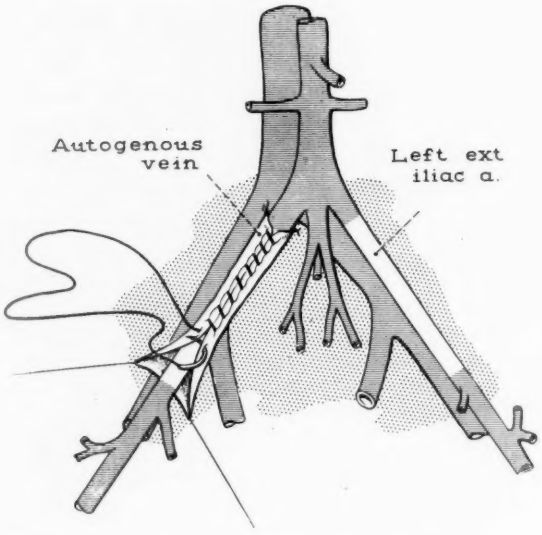

Fig. 1. Operative site. Each external iliac artery, from aorta to deep femoral artery, was stripped of as much adventitia as possible, and a cuff of autogenous vein was sutured about one of the arteries.

only interrupts the intrinsic vasculature of the wall, but also disturbs the innervation. That interruption of the nerves to an artery results in anatomic changes in the arterial wall has been claimed[11, 17, 25] and denied.[4, 6, 13]

None of the experiments cited provided information from which the effect of simple surgical removal of adventitia from a relatively long segment of artery could be deduced. Our purpose was to gain this information. In order to obtain, at the same time, information concerning the pathogenesis of whatever changes might be produced, we attempted to delay revascularization of the walls of the freed arteries by suturing a cuff of autogenous vein, endothelial surface outward, about one of the external iliac arteries in each animal (Fig. 1). Autogenous veins have been used surgically to avert penetration by granulation tissue in operations for repair of tendons[12] and reconstruction of nerves.[15] They were expected to function similarly in our studies.

PROCEDURES

In 14 mongrel dogs anesthetized with pentobarbital sodium, the aortic trifurcation was exposed through a midline lower abdominal incision. The pelvic peritoneum overlying the external iliac artery on each side was incised. Each external iliac artery, from the aortic trifurcation to near the origin of the deep femoral artery, was stripped of as much of its adventitia as possible by blunt and sharp dissection. The length of the artery thus freed varied with the size of the dog and ranged from 4 to 7 cm. Preliminary dissection in anesthetized dogs (which is not discussed further in this report) indicated that a thin layer of adventitia, approximately 0.1 mm. thick, must remain on the external iliac artery. Attempts to remove this inner layer of adventitia invariably tore the artery. In each of the 14 dogs to be described in this report, a meticulous attempt was made to dissect away adventitia to the point of impending arterial rupture. The dense connective tissue of the adventitia was teased off with aneurysmal needle, fine forceps, and scalpel until the surface texture, increased translucence, and flexibility of the arterial wall indicated that further dissection would be hazardous.

An 8 cm. length of external jugular vein was removed through an incision in the neck, and the ends of the vein left in place were ligated. The excised vein was opened longitudinally and was then sutured, intimal surface outward, about a dissected external iliac artery in each dog. A continuous suture with No. 5-0 arterial silk was used. Each end of the sleeve of vein was anchored to adjacent tissue with 2 silk sutures. The opened autogenous vein, stretched to surround the artery, formed a snugly fitting sleeve over a 3 to 4 cm. length of that vessel.

All operations were performed aseptically. No anticoagulants were administered and no special postoperative care was provided. The dogs were fed the usual balanced kennel ration.

Arteriograms were made in 4 dogs for the purpose of visualizing the terminal aorta and its branches. Radiopaque medium was injected through a polyvinyl tube that had been inserted into the aorta via a common

carotid artery to a point immediately proximal to the aortic trifurcation. The pertinent arteries were demonstrated by arteriography 6 months after operation and again 18 months after operation in 2 dogs, and 12 months after operation in another 2 dogs.

The dogs were killed by exsanguination under pentobarbital sodium anesthesia after postoperative intervals of 10 days (2 dogs); 20 days (1 dog); 30 days (1 dog); and 2, 4, 6, 12, and 18 months (2 dogs at each time). Immediately after death the distal portions of the aorta and the external iliac arteries were isolated from the rest of the arterial system by ligatures. They were perfused in situ for 4 hours with Bouin's fluid at a pressure equivalent to 90 mm. Hg. This procedure fixed and hardened the arteries in a configuration which more closely approached that in the living animal than does the usual, simpler method of placing an excised segment of artery in fixative. After excision and further fixation, the arteries were sectioned at 3 levels for histologic examination. Morphologic features of the right and left external iliac arteries from each experimental animal were compared with each other and with similarly perfused and prepared external iliac arteries from 14 healthy control dogs of similar size. The experimental dogs ranged in weight from 7.2 to 14.4 kilograms, and their average weight was 9.7 kilograms. The control dogs ranged in weight from 7.0 to 16.8 kilograms, with an average weight of 12.0 kilograms.

RESULTS

In 3 of the 4 dogs in which the experimental vessels were visualized roentgenographically, the external iliac arteries that were surrounded by autogenous vein had diameters slightly smaller than the opposite uncovered arteries (Fig. 2).

Examination of histologic sections showed that a layer of adventitia approximately 0.1 mm. thick had remained on each dissected artery. Vasa vasorum which had either escaped removal or had re-formed since the operation were demonstrated within or immediately adjacent to the remaining layer of adventitia of all arteries, including those which had been surrounded by vein after dissection.

A collar of collagenous connective tissue with sparsely scattered elastic fibers rapidly formed about the dissected arteries. Collagenous tissue was especially prominent between the autogenous veins and the arteries they surrounded (Fig. 3). This layer of connective tissue had apparently caused the minor reduction in the lumen size of vessels sheathed by vein. In arteries examined after postoperative intervals longer than 1 month, the coat of collagenous tissue appeared hyaline and relatively acellular. The thickness of the collagenous neoadventitia varied greatly from dog to dog and was independent of the duration of the experimental interval. Elastic fibers, which are prominent in the adventitia of the dog's external iliac arteries, formed a minor part of the neoadventitia.

No consistent abnormalities of the intima or of the media were found in histologic sections of the experimental arteries. No differences could be demonstrated between the intima or media of the vessels from which adventitia had been removed and those of the vessels which had, in addition, been surrounded by autogenous vein.

The media in each artery appeared structurally intact except that, in an isolated

Fig. 2. Aortogram made 12 months after removal of adventitia from both external iliac arteries in a dog. Right external iliac artery, which was surrounded by a sleeve of vein, is slightly narrower than left.

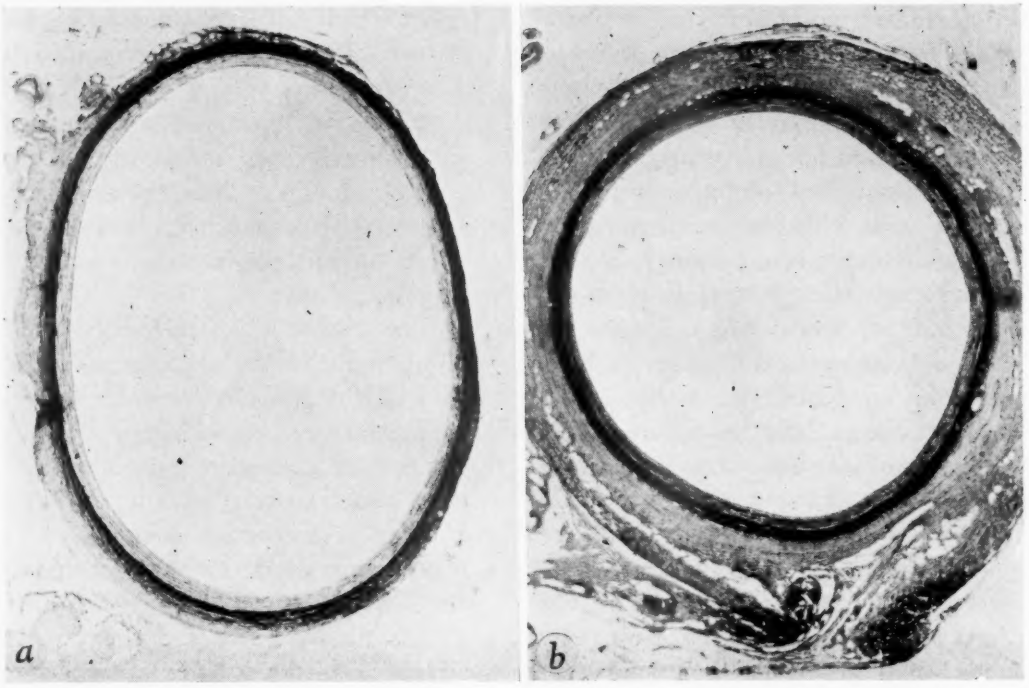

Fig. 3. Cross sections of left (*a*) and right (*b*) external iliac arteries of a dog 12 months after surgical removal of adventitia (*a*) and suture of a sleeve of vein about one of the arteries (*b*). These are same arteries outlined by aortogram in Fig. 2. Difference in luminal dimensions, barely apparent in aortogram, has been exaggerated by fixation. Note heavy collagenous envelope surrounding artery at right. No abnormality of media or intima is evident. (Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain; ×20; reduced 1/7.)

focus in each of 2 arteries, connective tissue had replaced smooth muscle. Each of these 2 defects was associated with a rupture of the adjacent inner elastic membrane and with disorganization and disappearance of elastic fibers in the media.

In order to determine whether a change had occurred in the mass of the media during the course of the study, planimetric measurements were performed and the average cross-sectional area of each artery was derived. Each of 3 histologic sections from each artery was projected at a magnification of 30 diameters. The media in the projected image of the artery was outlined on paper, and its area was determined by planimetry. The mean cross-sectional area of the media in the experimental arteries was 1.196 mm.^{2} The mean cross-sectional area of the media in the control arteries was 1.156 mm.^{2} The slightly larger cross-sectional area in the experimental arteries was not significant ($p = 0.38$). There was no significant difference between the cross-sectional area of the media of arteries from which adventitia had been removed (1.218 mm.^{2}) and those which had, in addition, been surrounded by autogenous vein (1.173 mm.^{2}).

The greater part of the intima was normal in all of the arteries, and the entire intima was normal in all histologic sections examined from 7 of the 28 experimental arteries from the 14 dogs (Fig. 4). Small focal lesions were observed in the intima of 21 vessels; the inner lining of these vessels otherwise retained its normal appearance, with a single layer of endothelial cells resting directly on the inner elastic membrane. Included among these focal lesions were breaks and reduplications in the inner elastic lamina, focal accumulations of collagenous connective tissue covered by endothelium and protruding into the lumen (Fig. 5), and shallow intimal crescents of proliferated connective

tissue. The largest of these crescents, which was present in a dog killed 4 months after operation, is shown in Fig. 6. This lesion, which contained both collagenous and elastic tissue, was located near a discrete foreign-body reaction which surrounded a silk ligature that had been used to tie off a small branch of the autogenous vein placed around the artery. Most of the intimal lesions were associated with disruption or reduplication of the inner elastic membrane. Fibrin was demonstrated by Weigert's method in one such lesion in an artery examined 10 days after removal of adventitia.

DISCUSSION

The focal nature of the intimal lesions found in this study and their frequent association with abnormalities of the inner elastic membrane led us to believe that these proliferations of connective tissue in the intima were the result of trauma incurred during surgical dissection of the adventitia. The presence of fibrin in 1 early lesion suggested that its origin was a small mural thrombus, perhaps at a site of minor injury. That proliferation of connective tissue occurs in the intima overlying tears in the inner elastic membrane and over traumatic lesions

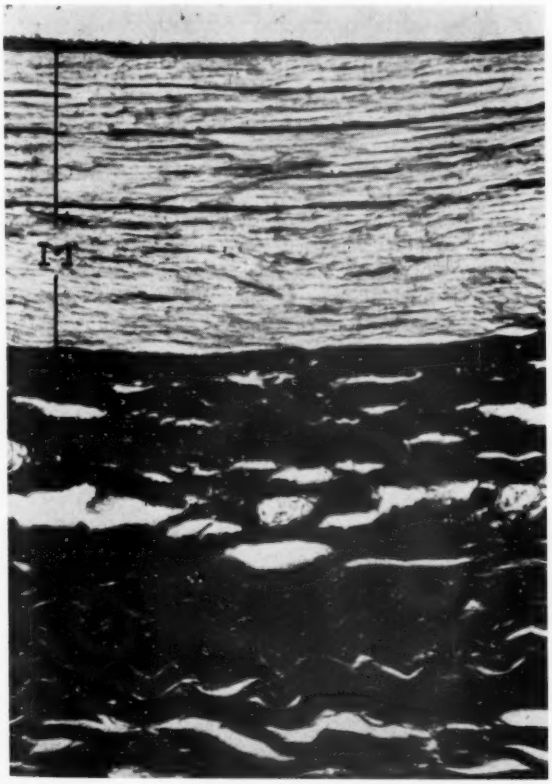

Fig. 4. Cross section of the external iliac artery of a dog 18 months after surgical removal of the adventitia. The lumen is at the top, adventitia is at the bottom, and the media is indicated by *M*. Nuclei of endothelial cells, which were flattened by perfusion of the vessel with fixative under pressure, are barely visible on the heavy internal elastic membrane. The intima and media appear entirely normal. Spaces which appear in the adventitia are fixation artifacts. (Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain; ×350.)

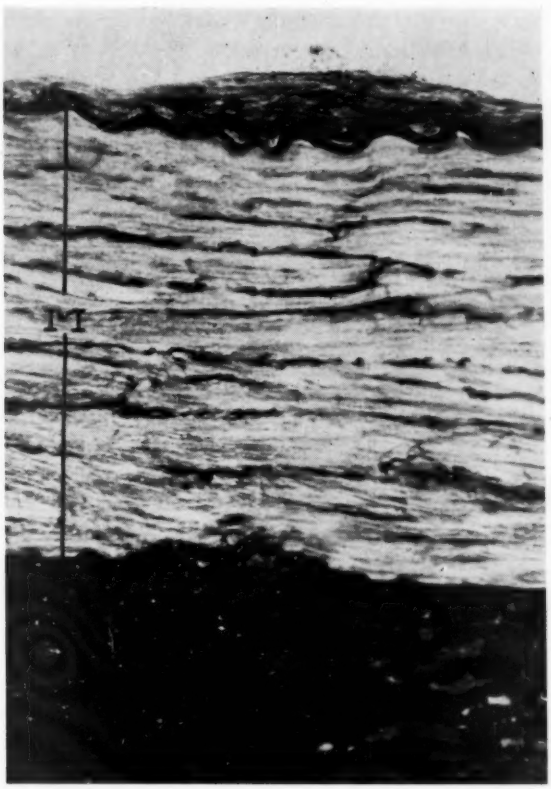

Fig. 5. Cross section of external iliac artery of a dog 12 months after surgical removal of adventitia. Lumen is at top, adventitia is at bottom, and media is indicated by *M*. Focal intimal lesion consists mainly of collagenous connective tissue. Fibrin was demonstrated in a similar lesion from a dog examined 10 days postoperatively which suggested that the lesion arose from a small mural thrombus. The inner elastic membrane is split at the base of the lesion. (Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain; ×350.) (From the Armed Forces Institute of Pathology, Washington 25, D. C.)

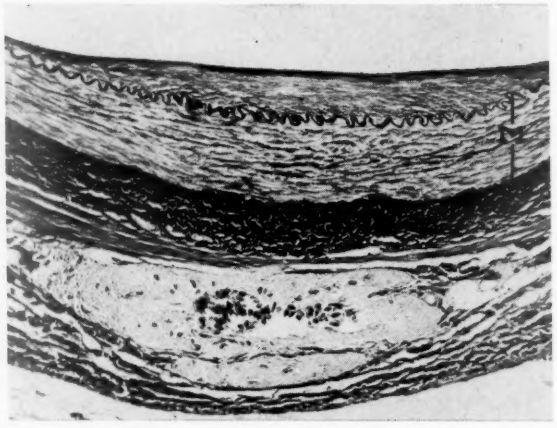

Fig. 6. Crescent of collagenous and elastic tissue in intima of external iliac artery of a dog 4 months after surgical removal of adventitia. Lumen is at top, adventitia is at bottom, and media is indicated by *M*. Intimal lesion overlies circumscribed foreign-body reaction about suture material near bottom of photograph. (Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain; ×70; reduced 3/10.) (From the Armed Forces Institute of Pathology, Washington 25, D. C.)

of the media is well known.[16, 21, 23, 26] Healing of small traumatic medial lesions may be so complete that the site of injury can later be identified only by persisting changes in the intima.

Deterling reported histologic studies of arterial autografts in which minor decreases in thickness of the media were observed in the graft. Those studies concerned carotid arterial grafts in dogs. It seems reasonable to suppose that complete, if temporary, disruption of the vasa vasorum which supply the grafted segment of artery had something to do with the loss of medial substance found by Deterling. The absence of this change and of all other consistent degenerative changes in the media of the experimental arteries observed by us may be interpreted as indicating either that (1) the surgical removal of adventitia left intact an adequate network of vasa vasorum to supply the needs of the artery wall or that (2) the metabolic needs of the walls of the arteries were supplied by diffusion from the lumen of the arteries and from surrounding tissue during the period, presumably short, required for re-establishment of an intact vasal network. In either case, the conclusion remains the same—the adventitia of the external iliac arteries of the dog may be reduced surgically to the point of impending arterial rupture without pathologic effect on the media or intima of the vessels.

SUMMARY

Meticulous surgical removal of as much adventitia as possible from the external iliac arteries in 14 dogs caused no consistent changes in the media or intima of those arteries during periods of as long as 18 months. Focal intimal and medial lesions which were produced could be attributed to the trauma of the procedure. Wrapping the dissected arteries with segments of autogenous vein to delay revascularization of the walls of the arteries had no pathologic effect except that, in 3 of 4 animals studied by aortography, arteries surrounded by vein were slightly smaller than arteries not surrounded by vein.

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Mucosal replacement of gastric explants by free autogenous grafts

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Previous reports have described studies of replacement of gastric mucosa by a nonsecretory epithelium.[1, 2] The application of split-thickness autogenous skin grafts to the denuded gastric wall in the pig was followed by variable success, which yielded an average survival of approximately 45 per cent of the grafted skin. Additional studies have been conducted to determine the factors that may influence the fate of these skin grafts. A wedge of the greater curvature portion of the stomach with an intact pedicle was employed as an explant on the anterior abdominal wall to visualize the events that follow mucosal stripping and grafting.

METHODS

Ten-week-old pigs which weighed 20 to 30 pounds were anesthetized intraperitoneally with Nembutal and maintained with an ether-oxygen mixture administered through an endotracheal tube. A split-thickness skin graft approximately 0.010 inches in thickness was obtained from the anterior abdominal wall of 5 pigs. Jejunal mucosa was obtained in 9 pigs by stripping the mucosa from a resected portion of the jejunum. Bladder mucosa was obtained by performing a cystotomy and stripping the transitional epithelium just above the muscularis of the urinary bladder in 3 others. The grafts were kept in a saline-soaked sponge until the gastric explant was prepared.

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The abdomen was entered through an upper midline incision, and a well-vascularized wedge of the greater curvature of the fundus measuring 4 by 8 cm. was excised. The gastric defect was closed with continuous inverting chromic catgut sutures and reinforced with interrupted sutures of fine silk. The explant with its intact vascular pedicle was transferred to the anterior abdominal wall. The incision was closed in layers with interrupted fine silk sutures about the vascular pedicle. The explant was secured to the abdomen at its margins and then stripped of gastric mucosa by blunt and sharp dissection just below the muscularis mucosa, a relatively avascular, filmy areolar plane (Fig. 1). Hemostasis was obtained by compression. The graft was applied and secured to the explant by interrupted fine silk sutures (Fig. 2). A bulky bolus dressing was utilized. The dressings were changed at 3- or 4-day intervals so that the progress of the graft could be observed and documented by photographs and histologic sections. Four explants were transferred into the subcutaneous tissues of the anterior abdominal wall in an attempt to secure successful jejunal mucosal grafts. In these instances the graft site was not disturbed for a period of 1 to 2 weeks.

RESULTS

Split-thickness skin grafts which covered the gastric explant demonstrated virtually complete survival in the 5 animals at the time of sacrifice 3 weeks after application.

The sequence of healing is demonstrated in Fig. 3. A smooth, pliable surface was obtained in each instance. Though there was no evidence of shrinking of the explant for the duration of this study, the histologic sections demonstrated a dense desmoplastic scar beneath the graft (Fig. 4). Nine grafts of jejunal mucosa were performed. Two pigs died from pneumonia 3 and 4 days after operation with the grafts still intact. Six grafts survived 7 to 12 days and then sloughed. The longest graft survival was 20 days in a pig in which the explant had been transferred into the subcutaneous position rather than onto the anterior abdominal wall.

Three preparations in which urinary bladder mucosa was applied to the denuded explant were unsuccessful. Two demonstrated loss of the graft in 3 days, while the third graft survived for 11 days and was then lost.

DISCUSSION

The replacement of gastric mucosa by nonsecretory epithelium with preservation of the storage and trituratory functions of the stomach would be a desirable adjunct to the surgical therapy of peptic ulcer disease. However, there are numerous obstacles to the practical solution of this problem. Among these has been the regeneration of gastric mucosa beneath and around the graft site in the intact stomach of some laboratory animals. This was solved in part by the use of the pig in which the mucosa can be completely stripped with ease beneath the muscularis mucosa.[2] Acid-secreting mucosa, however, remains at the margins and continually bathes the graft with gastric juice. Constant motion at the graft site from the muscular contractions of the gastric wall tend to displace the grafts as well. Attempts to secure immobilization by use of a bolus, multiple fixation sutures, or distention of the gastric lumen have met with indifferent results.

The gastric explant as employed in this study has afforded an opportunity for direct-vision study of grafts applied to a well-vascularized immobile surface, free from the effects of gastric juice. A uniform, pliable, nonsecretory surface was insured when split-thickness skin grafts were employed. The success of these preparations confirms the role of immobilization of the graft site, a principle which is basic to successful grafting in all areas of the body. The significance of establishing an acid-free environment demands additional study.

A third factor to be considered is the effect of shrinkage of the desmoplastic scar beneath the graft. Within the time period of this study, which was of short duration, there was no gross shrinkage of the explant. Longer periods of observation are necessary to determine the effects of contraction as the underlying scar in the graft bed matures.

Attempts to graft the denuded explant with epithelium other than skin were unsuccessful. Lippman[3] observed in his prep-

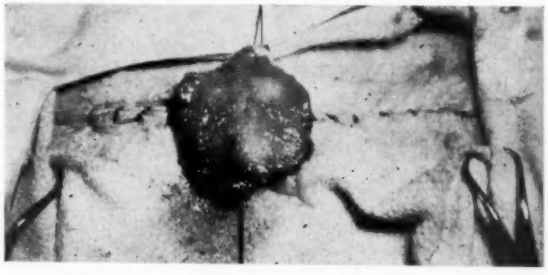


Fig. 1. Appearance of the gastric explant which has been formed from a section of the greater curvature of the stomach and has been stripped of the gastric mucosa.

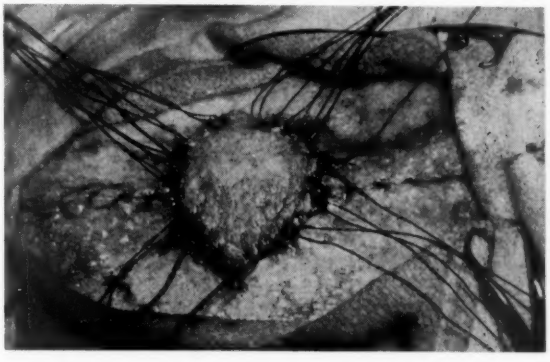
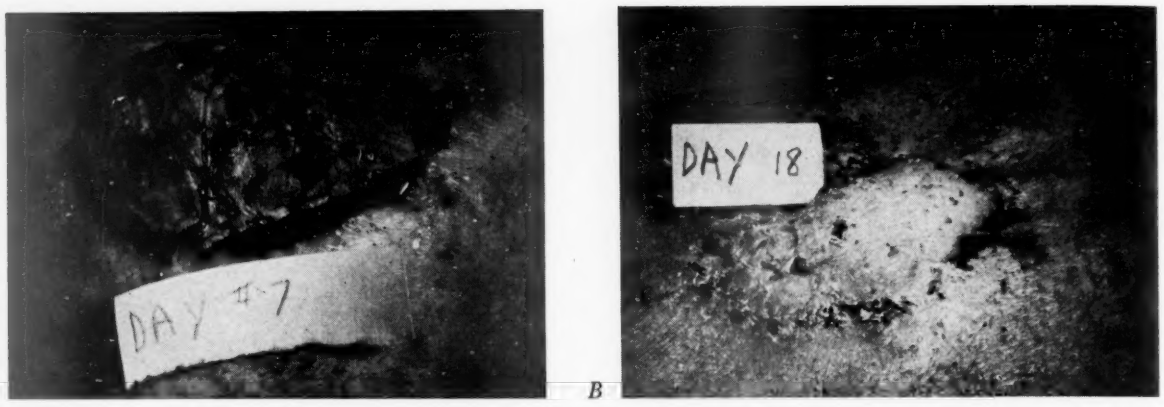
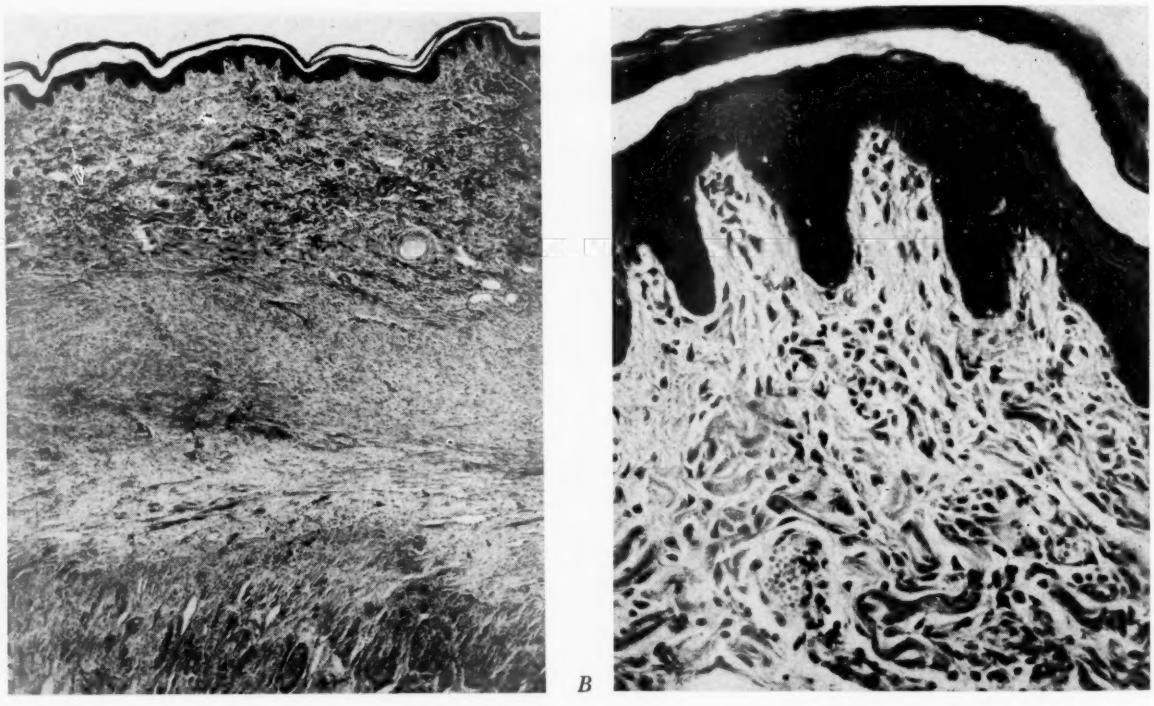


Fig. 2. A split-thickness autogenous skin graft has been applied to the gastric explant in a pig and the margins of the explant have been sutured to the skin of the anterior abdominal wall.



A B

Fig. 3. *A*, Appearance of the grafted explant 7 days after the application of the skin graft. *B*, The skin graft has taken well 18 days after application.



A B

Fig. 4. *A*, Photomicrograph of section through the grafted explant 3 weeks after application of skin (low-power view). *B*, High-power view of the same section which demonstrates the formation of scar tissue beneath the graft. (Reduced 3/10.)

arations that the jejunal mucosa for the most part undergoes necrosis, with only a few cells remaining as histologically recognizable jejunal mucosa. We judged the failure of our preparations by the gross appearance of slough, and it may be that further observation of these grafts would have demonstrated subsequent regeneration.

The failure to secure successful bladder mucosal grafts cannot be explained at this time. A chronic inflammatory reaction was observed in the explant in each instance which may have led to the rejection of the graft. The etiology of this response, however, remains obscure.

The excellent surface obtained with skin, in addition to its availability, has encouraged its continued use as an appropriate epithe-

lium for relining the stomach. The problem centers on a method for insuring a uniform "take" of a large grafted surface in the intact stomach. The method presented in this paper was used in an attempt to study one of the factors that appears to be involved in the problem.

SUMMARY

Free autogenous grafts have been applied to a gastric explant of the pig's stomach denuded of its mucosa. An acceptable epithelial surface of split-thickness skin was obtained. Jejunal and urinary bladder mucosal grafts were unsuccessful.

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Artificial respirator for dogs

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Unless respiration is assisted by manual aid or a mechanical device, the administration of an anesthetic such as Nembutal will produce hypoventilation and may reduce the oxygen saturation of arterial blood to approximately 75 per cent. An artificial respirator is required not only for open-chest operations, but also in all experimental procedures in which organ function must be studied without the interference of anoxemia. Experiments on 10 dogs with a newly devised positive-pressure respirator have resulted in arterial blood oxygen saturation of nearly 90 per cent. Besides its effectiveness in improving ventilation and combating anoxemia, the apparatus is simple to operate and easy to control.

DESCRIPTION

A motor-driven electric timer (Fig. 1, *TM*), which has a 2-second-on, 2-second-off cycle, activates relay RI_1.

During the 2-second-off cycle a negative grid bias of 4 V. is applied to thyratron tube *(V)*, keeping it cut off. In the 2-second-on position, RL_1 allows the charged capacitor (C_2) to discharge through R_2, R_3, R_4, or R_5, resulting in a time constant of ½, 1, 1½, and 2 seconds, respectively. The discharge of C_2 lowers the voltage on the grid of *V*, causing the tube to conduct and energize RL_2. When RL_2 is energized, RL_3 is de-energized, which in turn activates the solenoid valve (SL_2), through which the dog exhales. The variable time lapse between the start of the on cycle of the timer and the conduction of *V* is the interval during which the solenoid valve, *SL*, is energized and the dog inhales (positive inhalation). The relationship between these cycles is shown in Fig. 2.

A laboratory air line is connected through a pressure regulator to the solenoid valves which, during the inspiratory cycle, allow

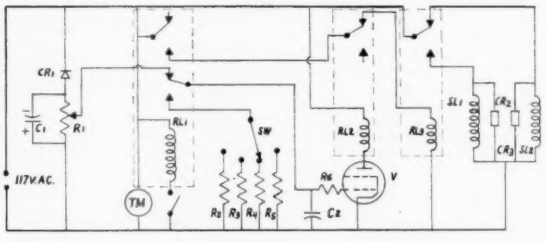

Fig. 1. Circuit diagram. CR_1, 150 Ma. selenium rectifier; CR_2, and CR_3, contact protectors, International Rectifier Corp., No. 56Y6P; R_1, 30 kohm, 4W potentiometer; R_2, 1 megohm ½ W resistor; R_3, 2 megohm ½ W resistor; R_4, 3 megohm ½ W resistor; R_5, 4 megohm ½ W resistor; R_6, 10 kohm ½ W resistor; C_1, 8 MFD 150 v. capacitor; C_2, 0.5 MFD 150 v. capacitor; *V*, 2 D 21 tube; *TM*, Industrial Timer Corp. model CMO 115/60; RL_1, Potter and Brumfield relay KRP11A; RL_2 and RL_3, Potter and Brumfield relay KRP5A; SL_1, V 5 D 6260C solenoid valve; and SL_2, V 5 D 1920S solenoid valve. The pressure regulator is Black, Sivalls and Bryson type 001-21.

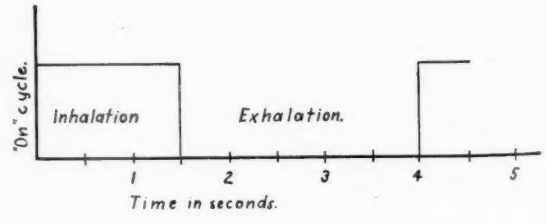

Fig. 2. Comparison of inhalation and exhalation time with SW in the 1.5 second position.

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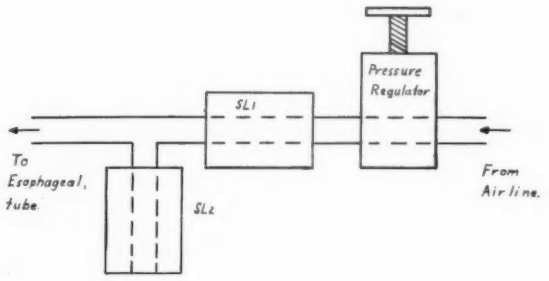


Fig. 3. Diagram of the air system.

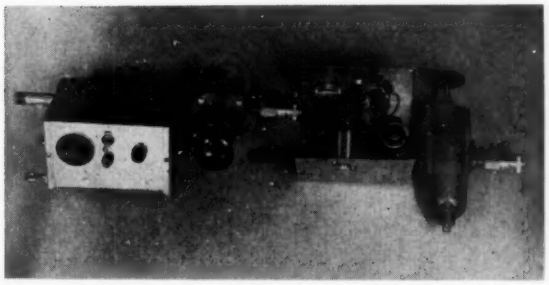
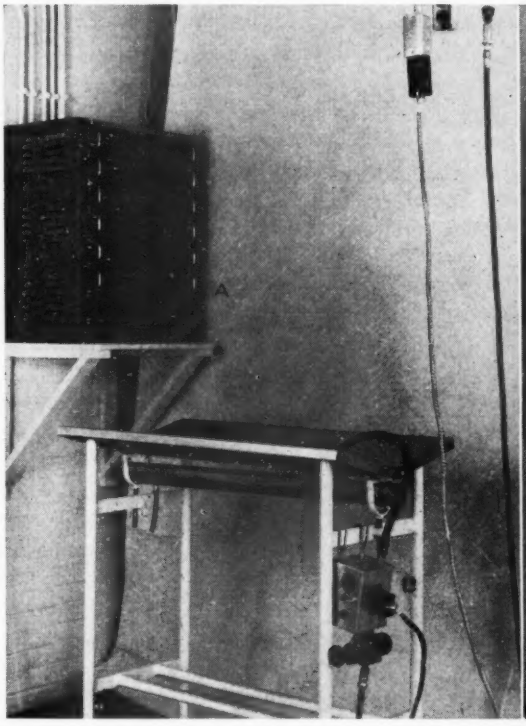
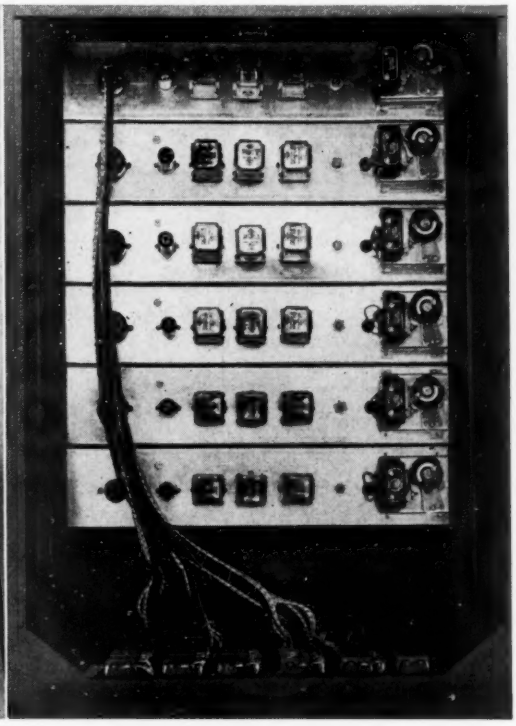


Fig. 4. Two remote units shown in an assembled and exploded view.





5 *6*

Fig. 5. *A,* Master unit containing 5 individual respirator units with 1 unit as a spare. *B,* Remote unit mounted to the operating table.

Fig. 6. Rear view of master unit which shows the electrical connections to the remote unit.

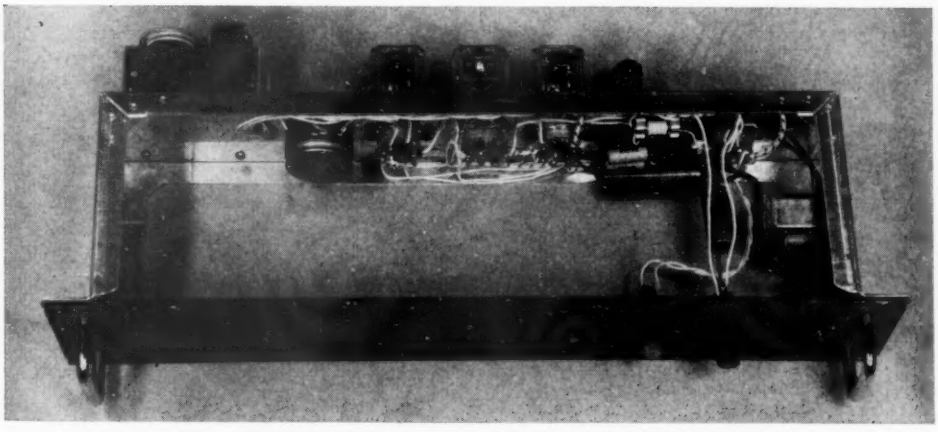


Fig. 7. View of individual rack-mounted unit.

the air to flow through an intertracheal tube to the lungs of the dog (Fig. 3). The large orifice of SL_2 allows complete exhalation. CR_2 and CR_3 are used for arc-suppression across the contacts of RL_3.

SPECIAL FEATURES

The 2 solenoid valves, the pressure regulator, the on-off switch, and the tap switch (for adjusting the inspiration time) are mounted in a small aluminum box (Fig. 4). This box may be attached to the operating table usually used for animals (Fig. 5).

Electrical connections to the master unit (Fig. 6) are made via a cable and plug. The air line is connected by a quick connect-disconnect coupling that allows fast disassembly and easy storage in a crowded laboratory. Plug-in type relays are used for quick and simple servicing. The unit shown in Figs. 5, *A*, and 6 accommodates 5 separate respirators with 1 for use as a spare. All channels are rack-mounted for ready removal (Fig. 7).

SUMMARY

The respirator described is a very simple yet reliable unit. It complies with the basic demands for 15 respirations per minute, a variable inspiration time (½ to 2 seconds), adjustable flow and pressure to the lungs, operational simplicity, and compactness. This unit can also be connected to a supply of oxygen or other gas mixtures.

The author wishes to thank Dr. Oscar Creech, Jr., Professor and Chairman of the Department of Surgery, Tulane University School of Medicine, for his cooperation in the development of this respirator. The suggestions of Professor James Cronwich of the Electrical Engineering Department of Tulane University concerning the preparation of this paper are gratefully acknowledged.

Anomalies of the lungs associated with congenital diaphragmatic hernia

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Structural defects in the closure of the pleuroperitoneal hiatus or the formation of the hemidiaphragm account for the majority of congenital diaphragmatic hernias in infants and children.[10] Invariably, some other embryologic abnormality is associated with these diaphragmatic defects.[3] The one commonly reported is the abnormal mesenteric attachment of the small and large bowel.[5, 8, 10, 19] However, the occurrence of another anomaly accompanying congenital herniation of the abdominal viscera into the thoracic cavity, namely the ipsilateral lung, has received little attention.[3, 20] While most reports of large series of patients treated operatively fail to mention the state of the lungs at operation, others, more recently, have stressed the high incidence of accompanying pulmonary hypoplasia.[3, 20] Another uncommon pulmonary anomaly, a "lower accessory lung" or "extralobar sequestration," has been seen to accompany congenital diaphragmatic defects both at autopsy and at operation.[9, 21, 22]

The case histories of 5 patients who had congenital diaphragmatic hernias with associated pulmonary abnormalities are reported. In 2 patients, pulmonary hypoplasia of the ipsilateral lung was seen at operation, while in the third, an unsuspected "extralobar sequestration" of the lung was found during repair of the hernia and was resected. In 2 other patients with congenital diaphragmatic hernias, in whom death occurred soon after birth, autopsies were performed. In one there was definite hypoplasia of the ipsilateral lung, while in the other there was nonpneumatization of both lungs. The variety and high incidence of pulmonary anomalies encountered with congenital diaphragmatic hernias and the importance of recognizing the presence of these anomalies prompted us to report these case histories and to review the literature on the subject.

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CASE REPORTS

Case 1. A 6 pound, 13 ounce baby girl was born at St. Alexis Hospital at 3:35 p.m. on Oct. 10, 1952. Respirations were spontaneous, but labored, and the baby was cyanotic. Breath sounds were absent on the left side of the chest. Roentgenograms were suggestive of the presence of a congenital diaphragmatic hernia on the left (Fig. 1). The baby was operated upon 48 hours after birth. A thoracoabdominal approach was utilized. All the abdominal organs, with the exception of the kidneys, descending colon, and rectum, were in the left hemithorax. The left hemidiaphragm was completely absent. The hernia was repaired with the left lobe of the liver used to close the communication between the pleural and peritoneal cavities. The edge of the liver was sutured to the endothoracic fascia. Repeated efforts during operation to expand the small and collapsed left lung were unsuccessful. At the time of closure, the left lung was completely collapsed against the medias-

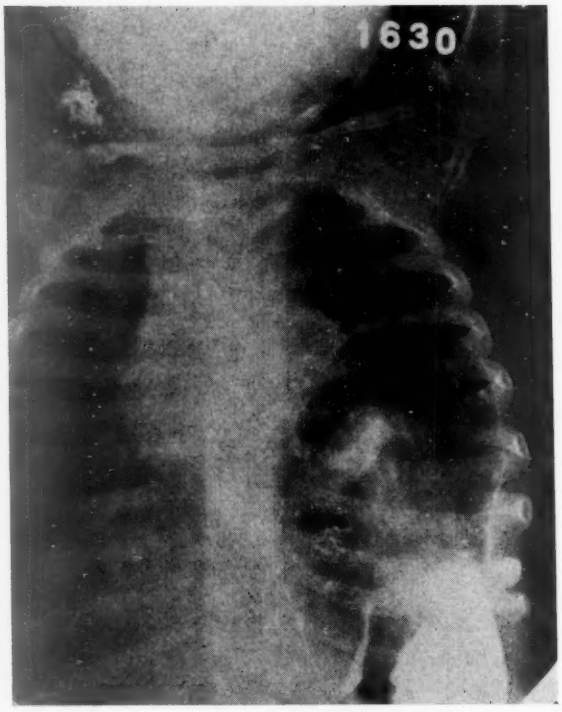

Fig. 1. Case 1. Preoperative chest roentgenogram after a barium swallow. Note the fundus of the stomach high in the left pleural cavity. The mediastinum is not displaced.

tinum. Postoperatively, the thoracotomy tube was attached for water-sealed drainage. Negative pressure was applied to the thoracotomy tube every 4 hours. Gradual expansion of the left lung took place. The thoracotomy tube was removed on the ninth day when roentgenograms of the chest showed the heart to be back in its normal position and both lungs to be aerating well. Eight years later the patient was doing well (Fig. 2).

Case 2. In December, 1952, a 24-hour-old, 7½ month premature infant was seen at Huron Road Hospital with signs of a right diaphragmatic hernia. Roentgenograms showed the stomach to be in the abdomen, but the small bowel and most of the colon were in the right hemithorax. The baby was operated upon through a right thoracoabdominal approach in the eighth intercostal space. The right hemidiaphragm was completely absent. There was difficulty in reducing the small intestine and colon back into the abdomen because of marked distention of the bowel. The right lung was completely collapsed and proved impossible to expand. The liver was sutured to the endothoracic fascia and effectively created a barrier between the two cavities. At the conclusion of the operation, cardiac arrest occurred and the heart failed to respond to cardiac massage and other measures. No other abnormalities were seen at autopsy.

Case 3. A 4 pound, 3 ounce male was born on Oct. 19, 1954, at Cleveland Metropolitan General Hospital. The baby cried spontaneously at birth, but was cyanotic and had shallow, irregular breathing. His condition deteriorated rapidly and he died a few hours after birth. At autopsy, the left hemithorax was found to contain the left lobe of the liver, cecum, terminal ileum, transverse colon, and spleen. There was a hernial sac present. Both lungs were completely collapsed, were reported as showing "nonpneumatization," and were probably hypoplastic (Fig. 3).

Case 4. The patient was a 3½-month-old male, who, on a routine examination in the clinic for an upper respiratory infection, was found to have a shift of the mediastinum to the right. Roentgenogram of the chest (Fig. 4) was suggestive of a left diaphragmatic hernia, and the patient was admitted to the hospital. He had been born at full term at this hospital on Nov. 1, 1958, and discharged 5 days later in good condition. He ate well and developed normally. On admission the baby was in no acute distress. There was a shift of the trachea and the mediastinum to the right, and the left side of the chest showed increased tympany and depressed breath sounds. A left thoracotomy was performed through the eighth intercostal space. A defect 5 cm. in diameter was found in the posterolateral portion of the muscular diaphragm. Through this defect protruded the stomach, spleen, transverse colon, and loops of small bowel. There were 2 normal looking lobes of the left lung. However, between the lower lobe and the diaphragm a third and apparently atelectatic lobe was seen. This felt like liver. The lobe received a systemic artery which entered through the posterior rim of the diaphragm. The vein drained below the diaphragm. There were no connections with the bronchial tree or pulmonary vessels. It was attached by alveolar tissue to the hilum. The mass was recognized as a "sequestrated lobe" (Fig. 5). The hernia was repaired after the sac had been opened and its contents easily reduced into the abdomen. The sequestrated lobe was then resected. The chest cavity was drained under a water seal. The patient made an uneventful recovery. The pathologic diagnosis of the resected tissue was "consistent with a sequestrated lobe of the lung" (Fig. 6). The patient was seen 20 months later and was doing well at that time (Fig. 7).

Case 5. A full-term male baby was born on July 5, 1959, at Cleveland Metropolitan General Hospital. The baby weighed 2,800 grams at birth, was cyanotic and flaccid with absent corneal reflexes, and was gasping. In spite of attempts at resuscitation, the baby remained cyanotic and died soon after birth. At autopsy, congenital absence of the left hemidiaphragm was found with

herniation of the stomach, pancreas, spleen, left lobe of the liver, most of the small intestine, cecum, and ascending and transverse colon into the left hemithorax. The left lung was hypoplastic (Fig. 8) and both lungs were atelectatic.

DISCUSSION

Pulmonary hypoplasia. The association of hypoplasia of the lung with congenital diaphragmatic hernias has been fully described as a postmortem finding by pathologists, but it was not until recently that the condition was recognized by the surgeon at operation. Potter,[14] in her book, *The Pathology of the Foetus and the Newborn,* writes, "Any condition that reduces the capacity of the thoracic cage early in foetal life will cause hypoplasia of the lung tissue. To develop normally the lung must have adequate room for growth, and growth will be inhibited in proportion to lack of space. The most profound reduction in size of the lungs occurs with diaphragmatic herniae, especially in the lung on the side of the diaphragmatic defect."

Campanale and Rowland,[3] in 1955, were the first to call attention to the presence of pulmonary hypoplasia in patients treated surgically for congenital diaphragmatic hernias. A moderate to severe hypoplasia of the lung was seen in every one of their 5 patients, 3 of whom were operated upon. However, a review by these authors of the surgical literature of the preceding 30 years, failed to disclose a single mention of this condition by the surgeon.[3] Gross,[3] after an extensive experience with congenital diaphragmatic hernias at Children's Hospital in Boston, could not recall noting at operation a single instance of definite hypoplasia of the lung and believed that it must be rare. Other surgeons who have recorded their observations on the lungs at operation indicate that a high percentage of the lungs were hypoplastic. Roe,[20] in 1956, reviewed the hospital records and autopsy protocols for all cases of congenital diaphragmatic hernias in 11 San Francisco hospitals over a period of 20 years. A total of 47 patients were studied and analyzed. Of 24 patients operated upon, in only 5 was satisfactory lung expansion described. In 10 cases the surgeon noted that the lung remained small and collapsed, in 1 there was partial lung expansion, and in the remaining 8 no comment on the lung was made in the operative note. Autopsy observations on 21 cases indicated that in size, the lung on the hernia side was often only a small fraction of the size of the contralateral lung, with an average weight ratio of 0.39. In 2 of our patients who were operated upon,[16] the appearance of the lungs was suggestive of hypoplasia. These lungs failed to expand in spite of strong positive pressure exerted through an endotracheal tube. In Case 2 there was a persistent pneumothorax postoperatively in spite of intermittent suction through a drainage tube, which indicated that the lung was hypoplastic and incapable of rapid expansion. There was definite hypo-

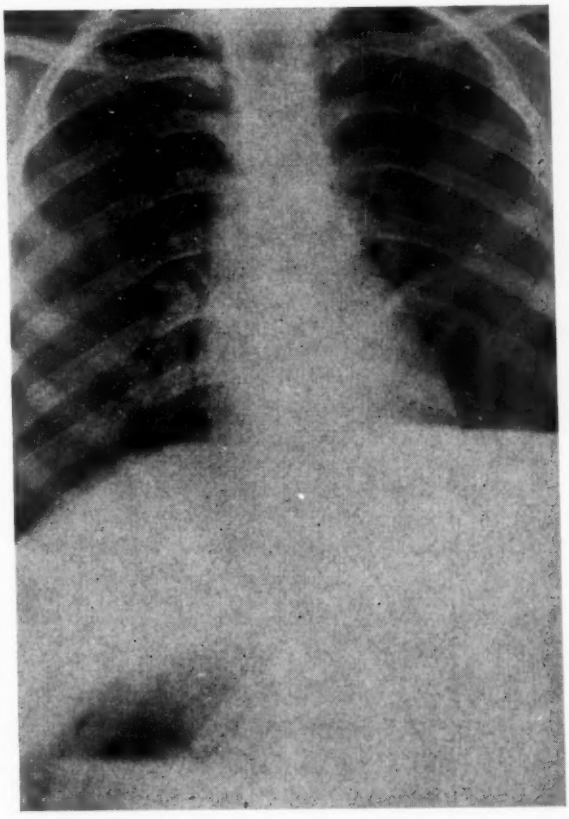

Fig. 2. Case 1. Chest roentgenogram 8 years postoperatively. The fundus of the stomach still appears higher than normal on the left side. The patient is asymptomatic.

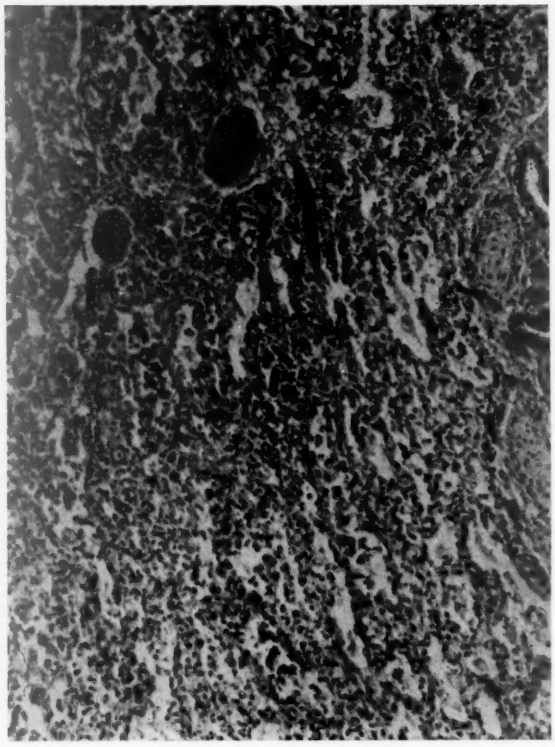

Fig. 3. Case 3. Photomicrograph of the left lung which shows nonpneumatization. Note few air-filled alveoli and atelectasis consistent with mild hypoplasia of the lung (×200; reduced ¼.)

plasia of the pulmonary tissue in the patient in Case 5 at autopsy.

Extralobar sequestration. This lung anomaly, also called "lower accessory lobe," is commonly associated with congenital diaphragmatic hernias. St. Raymond and coworkers,[21] who analyzed all cases reported in the literature up to 1956, found several characteristics common to all. These anomalies consisted of masses of lung tissue in various stages of differentiation occurring in the thorax, lower neck, or upper abdomen and not connected with the normal respiratory tree. They were not incompatible with life and were much more common on the left side. The presence of an associated defect of the diaphragm, predominantly of the left side, was not an infrequent finding. Except for an occasional rare case, the blood supply of the lower accessory lung was not associated with the pulmonary vascular system, but was derived from the aorta.[21] The venous drainage was usually to the hemiazygous system.[17] Concerning the genesis of this embryonic defect, the "traction" theory advanced by Pryce and colleagues[17] in 1947 adequately explains the developmental anomaly. In brief, they consider the abnormal blood supply of the "accessory lobe" to be the first abnormality, and sequestration to be an accidental complication. Abnormal arteries from the dorsal aorta on one side or the other of the embryo "capture" one or more "bulbous" tips of the developing lung tree. If such a "bulbous" tip of lung tissue survives, it becomes a "sequestrated" lobe. This same theory is advanced by these authors to explain the association of sequestrated lobes and diaphragmatic defects, and even the latter abnormality when present alone. According to this theory, congenital diaphragmatic defects are also the result of ischemic traction on abnormal embryonic arteries which run from the developing diaphragm to the developing lung. These are more common toward the pulmonary ligament on the left side. If a detached tip of lung tissue survives, the defect is then associated with a sequestrated lobe. Most diaphragmatic defects are not associated with sequestrated lobes because of the low survival rate of the detached pulmonary element.[17] In 1932, Guy and Rand[9] collected 34 cases of accessory lobe of the lung. Of these, 7 were associated with a congenital diaphragmatic hernia. They added an eighth case of their own. In 1947, Valle and White[22] collected 37 cases of lower accessory lungs. Of these, 29.7 per cent were associated with congenital diaphragmatic hernias. St. Raymond and his group,[21] in 1956, stated that about 40 cases of lower accessory lung had been reported. The majority of these cases were found at autopsy. St. Raymond reported the first case in which a patient with congenital diaphragmatic hernia associated with an accessory lobe was successfully operated upon. In 1947, Valle and White[22] had operated upon a similar patient who did not survive. Both patients were children with congenital diaphragmatic hernias in whom no lung anomalies had been suspected preopera-

tively. On exploration through a thoracic incision the accessory lobes were encountered and were resected. Our patient in Case 4 constitutes the third to be operated upon and the second reported to have survived. Similarly, in this case no lung anomaly was suspected preoperatively, and the condition was discovered at operation.

Other authors have reported the cases of patients with lower accessory lobes associated with other types of diaphragmatic defects. Claman and Ehrenhaft[4] described a patient 64 years old with an extralobar sequestration associated with a posterolateral weakness of the diaphragm. Berman[2] reported 2 cases of extralobar sequestration associated with "eventration of the diaphragm."

It thus appears that the lung anomalies, hypoplasia and extralobar sequestration, are more commonly associated with congenital diaphragmatic hernias than previously suspected. These anomalies have eluded the surgeons for several reasons. The first and most important reason may well be the great respiratory distress experienced by the newborn infant with a congenital diaphragmatic hernia in whom 1 lung is severely hypoplastic and the other compressed. Such patients rarely reach a surgeon and, understandably enough, the lesion has for a long time been not a surgical, but a postmortem finding. In Potter's[15] experience, only 1 infant with a diagnosis of congenital diaphragmatic hernia at the Chicago Lying-in Hospital survived more than 6 hours. Nevertheless, the experience of many surgeons[1, 5, 7, 10, 11, 19] has shown that not only can these babies be saved if operated upon soon after birth, but that a hypoplastic lung in the presence of a congenital diaphragmatic hernia is not incompatible with many years of life. Rakover and associates[18] reported the case of an 11-year-old boy with a congenital diaphragmatic hernia in whom the ipsilateral lung was quite hypoplastic and hardly filled one-third of the chest cavity when inflated by pressure at operation. The second cause for the lack of the surgeon's awareness of these associated

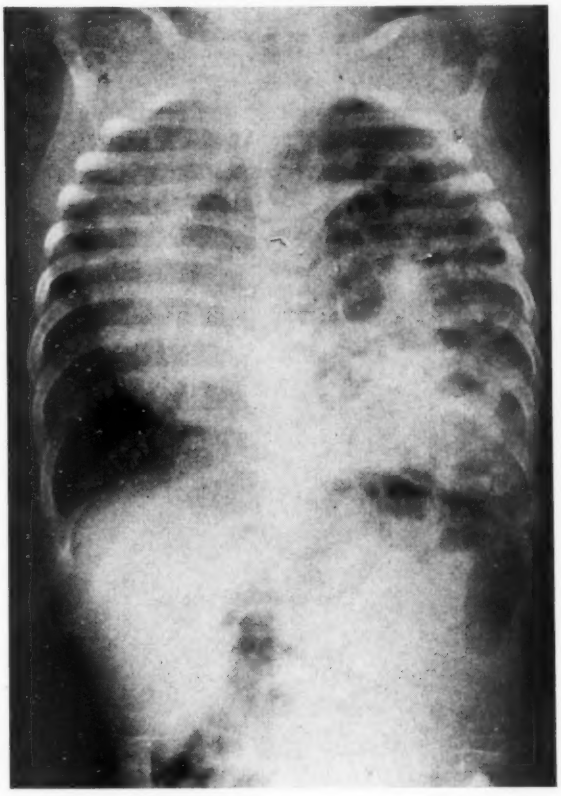

Fig. 4. Case 4. Preoperative posteroanterior chest roentgenogram which shows that the mediastinum is markedly displaced to the right and loops of the small bowel are filling the left hemithorax.

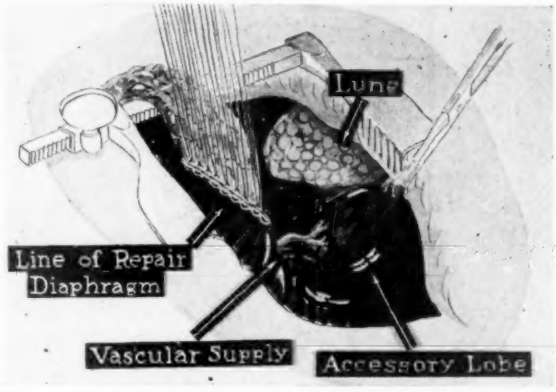

Fig. 5. Case 4. Drawing which shows the findings at operation (transthoracic approach). The suture line in the dome of the diaphragm is shown with the excess tissue from the redundant sac. The "extralobar sequestration" is shown with its vascular supply passing through the posterior rim of the diaphragm.

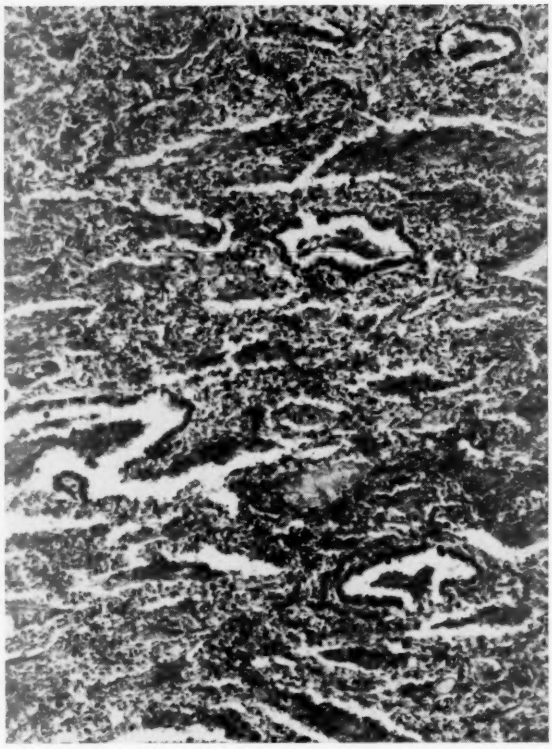

Fig. 6. Case 4. Photomicrograph of the resected tissue which shows cuboidal cell–lined air passages and structures like alveoli filled with blood, consistent with a "sequestrated lobe" of the lung (×200; reduced ¼.)

Fig. 7. Case 4. Postoperative roentgenogram taken 20 months later which shows a normal thoracic cavity with a normal diaphragm.

pulmonary anomalies is their failure to notice and record the presence of abnormalities of the lungs during operation. It is possible that since the majority of surgeons utilize the abdominal approach for the repair of this type of hernia,[5, 8, 10, 19] the exact state of the lungs and thoracic contents at operation remains unknown. A moderately hypoplastic lung or an extralobar sequestration may go unnoticed because the abdominal exposure precludes good visualization of the thoracic cavity.

According to Campanale and Rowland,[3] hypoplastic lungs present an anatomic and physiologic abnormality different from that of a collapsed but expandable lung. It is thus important for the surgeon not only to be aware of its frequent occurrence but also to recognize the condition when present. Forceful attempts to expand the lungs during operation with positive pressure through an endotracheal tube may be quite harmful and even fatal.[20] Furthermore, such efforts are rarely, if ever, rewarding since the hypoplastic lung is incapable of rapid expansion, as seen in 2 of our cases. The injudicious application of strong intrapleural negative pressure in an effort to "bring out the lung" can also lead to harmful effects.[3] In the presence of a poorly expandable hypoplastic lung the complete withdrawal of air will produce a mediastinal shift with cardiorespiratory embarrassment. Infants with this combination of defects have so limited a cardiorespiratory reserve to begin with that the superimposition of this added handicap can result in catastrophe.[3] It is advised that the intrapleural pressures be adjusted according to over-all clinical evaluation of cardiorespiratory function and not according to roentgenograms. Closed underwater drainage will assure gradual re-expansion of the lung to the extent possible.[3] Roe and Stephens[20] advocate intermittent gentle positive pressure to aid alveolar aeration and tracheostomy to reduce dead space in some cases. They also use closed sealed drainage to protect against tension pneumothorax and to promote gentle and gradual expansion of the hypoplastic lung.

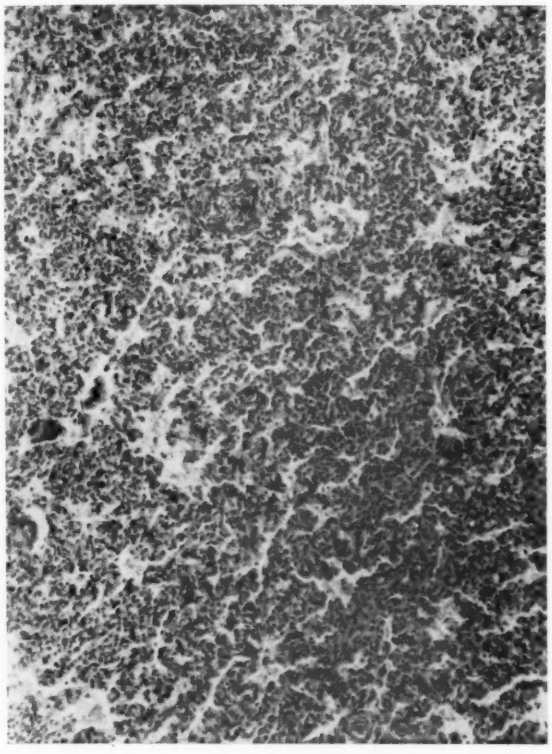

Fig. 8. Case 5. Photomicrograph of the left lung which shows hypoplasia. Note the poor degree of alveolar differentiation (×200; reduced ¼.)

An extralobar sequestration accompanying a congenital diaphragmatic hernia should be resected. Such a lobe can be the seat of inflammation.[6]

The routine use of a thoracic approach for the repair of congenital hernias allows easy detection of any lung anomaly. The thoracic approach has been previously advocated by Koop and Johnson.[12] These authors give as their reasons for this the immediate respiratory relief experienced by the baby on opening of the chest and relief of the intrathoracic pressure, the ease of repair of the defect, and the excellent exposure. An additional reason, in our opinion, is the high association of pulmonary malformations with these hernias. A thoracic approach affords the best opportunity for detecting and managing these abnormalities.

SUMMARY

1. The cases of 5 patients with congenital hernias through defects in the diaphragm associated with (1) hypoplasia of the lung and (2) extralobar sequestration are reported.

2. The association of pulmonary anomalies with such congenital hernias is more common than suspected. The literature is reviewed.

3. The thoracic approach for the repair of these hernias affords an opportunity for the detection and treatment of either lung anomaly and intelligent handling of the situation in the postoperative period.

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Management of recurrent or metastatic Wilms' tumor

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Much has been written about Wilms' tumor since the first case was described 150 years ago.[14] The bulk of the literature concerns the primary neoplasm, its embryologic derivation, signs and symptoms, histopathologic features, treatment, and end-result studies. With improvement in the methods of diagnosis and treatment, the survival percentages have correspondingly increased. The improved mortality rate permits the survival of patients with recurrent or metastatic disease who previously would have died of the primary tumor or its therapy. This increasingly important phase of the disease has received little attention.

The 28 cases of Wilms' tumor diagnosed in the Liverpool area during the last 12 years have been reviewed. Seven of the patients had recurrent or metastatic disease which was managed with apparent success so far as eradication of the neoplasm was concerned. Although only 4 of these are long-term survivors, the principles employed for the management of metastatic Wilms' tumor might be used to draw analogies applicable to the problem in general. For these reasons, a closer scrutiny has been made of this particular fraction of the group.

MATERIAL

The pertinent data concerning the 28 patients with histologically confirmed Wilms' tumors are recorded in Table I. The patients have all been drawn from Liverpool and its environs over the past 12 years. None of the cases has been previously reported except the 2 instances of bilateral neoplasm.[17] One of these patients has now survived 6 years free from known recurrence of the disease. Of the 9 patients who survived longer than 2 years, 4 had only the primary neoplasm and were treated for it alone. The remaining 5 patients were considered hopeless at one stage or another of the disease. Three of these are discussed at length (Cases 1, 2, and 3) and one of the others is the survivor with bilateral neoplasm alluded to previously.

In the light of the current concept that the younger the patient at diagnosis and treatment of the neoplasm, the better the prognosis, it is of interest to observe from Table I that the average age of the 2-year survivors in this series was slightly greater than that of the patients as a whole. This curious fact is not explained, except perhaps by our use of more aggressive therapeutic approach in children with recurrences than might be thought justified at other centers.

Treatment of the primary lesion, which has been largely standardized in this series, is summarized in Table II. It consists of extirpation of the neoplasm followed by deep roentgen therapy. The radiation is given during the first postoperative month to the dosage of 3,500 tumor roentgens delivered to the tumor site. In the patients treated during the past 18 months, the abdominal cavity has been irrigated with Clorpactin XCB at the conclusion of the operation, and actinomycin D has been given intravenously.

The following are the case reports of the 7 patients previously mentioned, in whom

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Table I. *Cases of Wilms' tumor in the Liverpool area, 1948 to present*

Cases	28
Location	
Right side	12
Left side	14
Bilateral	2
Palpable mass	26
Hematuria	
Gross	5
Microscopic	9
Untreated	2
Dead of neoplasm	12
Dead of other causes	3
Alive	11
2-year survivors	9 of 21 (42.8%)
Average age at diagnosis	
Entire series	3 yr., 1 mo.
2-year survivors	3 yr., 5 mo.

Table II. *Standard treatment of Wilms' tumor (primary)*

Surgical
Semiemergent, transabdominal approach, early ligature of vessels, en bloc node dissection
Irradiation
Begun in first postoperative week, dosage 3,500 r administered over 3 to 4 weeks
Recent adjuncts
Clorpactin used in surgical field and actinomycin D given intravenously, 15 mcg. per kilogram per day for 5 days

recurrent or metastatic neoplasm was successfully managed.

CASE REPORTS

Case 1. L. G. was an 8-year-old boy who had undergone resection of a huge Wilms' tumor on the left side. The primary tumor measured 22 by 13 by 12 cm., and weighed 1,650 Gm. No "spill" occurred during en bloc removal of the tumor and the grossly enlarged periaortic lymph nodes. The nodes were as large as 6 cm. in diameter. On histologic examination the neoplasm was found to be anaplastic and showed venous and lymphatic invasion. The regional lymph nodes were uniformly replaced by tumor. The patient was given the routine postoperative deep roentgen therapy. He is clinically well and chest roentgenographic examination showed him to be normal 7 years later.

A technical tour de force, this case illustrates the gratifying result which meticulous en bloc dissection and irradiation can achieve even with an extremely large tumor and regional metastases. This neoplasm, highly malignant on both clinical and histologic grounds, has been eradicated and the patient apparently cured.

Case 2. S. S. was a 4-year-old girl who was treated for a Wilms' tumor of the left side in the previously described manner. Four months after operation a chest roentgenogram showed rounded densities in both lung fields compatible with metastatic neoplasm. These disappeared after a course of deep radiation therapy (3,000 r) administered to both lung fields. Except for mild exertional dyspnea, the patient is well 12 years after treatment. She menstruates regularly and has grossly normal secondary sex development.

The case illustrates apparent cure of widespread pulmonary metastases by irradiation alone. Patently beneficial was the careful shielding of gonadal tissue while the patient was undergoing extensive roentgen therapy to the primary tumor site.

Case 3. F. M. was a 6-year-old girl who underwent resection of a Wilms' tumor of the right side. The tumor was dissected away from the inferior vena cava, which was displaced medially by neoplasm. The adrenal gland and periaortic lymph nodes were removed en bloc. Postoperative routine roentgen treatment was given. Four months later a pelvic mass was palpated, and chest roentgenogram showed rounded opacities in the lower lobes of both lungs. These lesions, compatible with metastatic neoplasm, disappeared after radiation therapy (3,000 r) to lung and pelvic fields. Six months after the operation apical pulmonary tuberculosis was diagnosed by chest roentgenogram and sputum culture, and was appropriately treated. The patient remains well 6 years after diagnosis of the neoplasm.

This case demonstrates effective management of multiple metastases by radiation therapy alone. The phenomenon of tuberculosis as a sequel to pulmonary irradiation is interesting, although the two are probably not causally related.

Case 4. E. D. was a 5½-year-old girl who had a nephroblastoma on the left side treated by en bloc resection and postoperative radiation. Histologic examination of the surgical specimen showed venous and lymphatic invasion. The preoperative chest roentgenogram was normal, but 1 month after operation repeat films showed infiltrates in the right lower and upper lung fields. These lesions disappeared after roentgen therapy (3,500 r) to both lungs. The patient died 4 months after operation. Postmortem examination showed pulmonary edema, bronchopneumonia, and pleural effusion. There was no gross or microscopic evidence of residual neoplasm.

This case demonstrates rapid dissemination of neoplasm which was presumably related to venous invasion. Ironically, the radiation therapy which

destroyed the pulmonary metastases also probably contributed to the death of the patient.

Case 5. P. F. was a 4-year-old girl who underwent resection of a Wilms' tumor on the left side. Postoperative roentgen therapy was administered in the routine manner. A metastasis was found in the left lung base by roentgenogram 10 months after operation which disappeared after radiation therapy (3,500 r) to the lung fields. The patient died 4 months after completion of this treatment. Postmortem examination showed bilateral pulmonary fibrosis with cor pulmonale and right-heart failure. No residual neoplasm was demonstrated by careful gross and microscopic studies.

This case again illustrates resolution of pulmonary metastases after roentgen therapy. However, the ultimate result was poor since death resulted from the cardiopulmonary changes which presumably occurred secondary to heavy pulmonary radiation.

Case 6. I. K. H. is a 5-year-old boy who underwent resection of a nephroblastoma on the left side at the age of 3 years. The tumor measured 18 by 11 by 8 cm., and weighed 1,080 Gm. The neoplasm had completely replaced the kidney and presented a variegated microscopic picture of anaplastic and mesenchymal elements. Routine radiation therapy was given. Eight months later chest roentgenogram showed a single "coin" lesion in the lower lobe of the right lung. In view of the noxious effects of pulmonary irradiation in the 2 previous cases, and since this metastasis appeared to be solitary, the lesion was resected. Histologic examination proved it to be a metastasis. The patient is clinically well and has no evidence of further pulmonary metastases by serial roentgenograms 20 months after resection of the lung metastasis.

This case has several interesting aspects. The primary tumor was large and presented a formidable technical problem at the time of extirpation. It contained most of the histologic variations which this pleomorphic neoplasm can assume. Finally, an apparently solitary lung metastasis was successfully managed by surgical means alone.

Case 7. P. P. was 7 years old when she underwent resection of a Wilms' tumor (right side) en bloc with the right renal vein, which contained a large tumor thrombus. The neoplasm had completely replaced the kidney, measured 18 by 10 by 6 cm., and on histologic examination appeared quite anaplastic. The aortic lymph nodes were microscopically free of tumor and the liver was grossly normal. Postoperatively the child was given a course of oral actinomycin D followed by the routine roentgen therapy. Ten months after operation a large epigastric mass was palpated. The patient's general condition was deteriorating. A second course of actinomycin D was administered, this time intravenously, with disappearance of the mass within 4 weeks. The patient remains well 9 months after treatment of the secondary tumor.

This case is an example of a clinically and histologically aggressive neoplasm which was effectively treated by a combination of operation, roentgen therapy, and chemotherapy. Of special interest is the response of the recurrent tumor to a second course of actinomycin D alone.

COMMENT

Management of primary Wilms' tumor is reasonably standardized, involving surgical extirpation and irradiation of the tumor area. Differences of opinion exist regarding the order in which these modalities should be used. Gross and Neuhauser[8] and others prefer resection prior to the radiation while Flocks and Kadesky[5] and others favor the reverse order. Kinzel and associates[9] and others suggest favorable results with preoperative irradiation, nephrectomy, and postoperative irradiation. The 2-year survival rates in these series do not indicate a clear superiority of any one approach as compared to the others. Judicious selection of cases for each sequence might be entertained. Preoperative irradiation might be used for huge lesions to increase the operability rate, technically facilitate the extirpation, and perhaps reduce tumor embolism incident to operative manipulation of the neoplasm. Operation followed by irradiation might then be reserved for tumors of more modest size and when histologic diagnosis is uncertain.

The incision used may be abdominal, thoracoabdominal, or loin (Nagamatsu). It should be large enough for adequate exposure of the lesion. The choice might be influenced by such factors as the surgeon's preference, size and location of the palpable mass, and so forth. Early ligation of veins and en bloc removal of nodes extending from the undersurface of the diaphragm or liver to the bifurcation of the great vessels with sacrifice of expendable contiguous structures involved by the neoplasm are time-honored principles of cancer surgery which are applicable in these cases.

A recent technical addition to treatment

of the primary neoplasm is irrigation of the surgical field with Clorpactin XCB at the conclusion of the operation, in hope of preventing growth of spilled tumor cells.[1, 7] The period of follow-up in these cases is so short that no conclusions can be drawn regarding its effect upon the incidence of local recurrence of neoplasm. No deleterious effects have been observed other than a possible increase in wound induration and inflammation. New experimental data presented by Cole and his group[11] suggest that a buffered 0.5 per cent sodium hypochlorite solution might be safer and more effective than Clorpactin for wound irrigation.

Another recent addition has been the intravenous administration of actinomycin D, 15 mcg. per kilogram of body weight daily for 5 days, the first fraction being administered concomitantly with surgical extirpation of the primary lesion. This chemotherapeutic agent has not been used long enough for any statement to be made regarding improvement in survival rates.

The objectives of this combination of therapeutic modalities is to remove all accessible gross neoplasm, destroy viable cancer cells spilled locally during surgical manipulation, reduce the viability of neoplastic emboli and, finally, destroy residual tumor in the local and regional areas by means of radiation.

Treatment of secondary manifestations of the disease is poorly documented and standardized. There is evidence of cure of metastases after roentgen therapy alone,[3, 6, 12] a presumption which the results in Cases 2 and 3 support. However, it is apparent that this method alone is not curative in most instances. Furthermore, heavy irradiation is noxious to normal as well as neoplastic tissue. Pulmonary tissue, one of the commonest sites of metastatic Wilms' tumor,[4] is particularly susceptible to irradiation damage.[15] Such damage contributed materially to the death of 2 patients (Cases 4 and 5).

Occasionally solitary and/or localized metastases in accessible sites can be resected[10, 16] as illustrated by Case 6. Stevens[20] suggests the use of intravenous chemotherapeutic agents while metastases are being resected. If this method can protect against tumor emboli dislodged by manipulation, it might enhance the role of surgical procedures in the management of secondary nephroblastoma.

Theoretically, systemically administered chemotherapeutic agents which are effective only against autonomously growing neoplastic tissue would be the ideal approach to the treatment of recurrent or metastatic neoplasm. Such agents are not yet available. Actinomycin D is one of the newer chemotherapeutic agents which is being tested. Reports which have thus far appeared suggest that its effect on tumor is unpredictable and palliation is measured in terms of weeks even in patients who respond favorably.[13, 18] Our own experience with this agent is limited, but as Case 7 illustrates, there is promise that palliation in at least some cases of metastatic Wilms' tumor can be anticipated. The side effects of actinomycin D treatment are annoying, especially when it is combined with roentgen therapy. They include accentuation of irradiation dermatitis, pancytopenia, alopecia, and inflammation of surgical wounds. To avoid the side effects of systemic administration, the concept of isolated perfusion of the pulmonary system[2] with actinomycin D seems attractive. If the leakage factor[19] could be reduced to a reasonably low level, thereby allowing actinomycin D to be given in greater concentrations than are now considered to be safe for systemic administration, this technique would be a worthwhile adjunct to the management of metastatic Wilms' tumor.

Multiple courses of treatment with actinomycin D can be given,[21] as shown by Case 7. It is believed that progressively less tumor effect will result with successive retreatments.[18, 21]

Wilms' tumors present formidable therapeutic problems. However, the course is as unpredictable as the histologic aspect is variegated. The criteria commonly associated with short survival, such as the occurrence of hematuria, large primary tumor, extensive regional node metastases, lymphatic

and/or venous invasion, histologic anaplasticity, older age of the patient, and the appearance of distant secondary lesions are not incompatible with long survival, as suggested by some of the cases discussed. Until the reasons for these inconsistencies are better understood, we must be patient and persistent in our approach to the problem and flexible in our attitude.

SUMMARY

A review is given of 28 patients with Wilms' tumors from the Liverpool area seen during the past 12 years. The 7 cases reviewed in detail are of particular interest because of recurrent or metastatic neoplasm which was managed with some success by operation, irradiation, or chemotherapy, singly or in various combinations. Recommendations are made and discussed for treatment of primary and secondary manifestations of the disease.

I wish to express my appreciation to J. S. Fulton, Medical Director of the Liverpool Regional Radiotherapy Centre, for allowing inclusion of his cases and providing details of roentgen therapy. Also, my thanks are extended to Miss I. Forshall and Mr. P. P. Rickham, Consultant Pediatric Surgeons to Alder Hey Children's Hospital in Liverpool, for permission to use their surgical cases and their advice and assistance in the preparation of this manuscript. Finally, I am grateful to R. H. Flocks, Professor and Head of the Department of Urology, State University of Iowa College of Medicine, Iowa City, Iowa, for constructive criticism of the manuscript.

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Coronary–internal mammary artery anastomosis in dogs

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Systemic-to-coronary artery anastomosis by various suture techniques[1, 2, 6, 8, 9] has met with only a limited degree of success in the experimental laboratory. The time required to perform suture anastomosis is such that some method of coronary perfusion is necessary to prevent ventricular fibrillation and infarction. Factors which limit successful anastomoses are the high acute surgical mortality rate and the propensity for vessels of this caliber, even in large dogs, to thrombose postoperatively. Intimal trauma, inherent in the performance of suture anastomoses, and the various techniques of coronary perfusion increase this risk of thrombosis. Carter and Roth,[4] stimulated by the success of nonsuture anastomotic techniques of Blakemore,[3] Weiss and Lam,[10] and Egdahl and Hume,[5] reported successful internal mammary–coronary artery anastomoses with polyethylene rings and a nonsuture technique. The average follow-up of their animals was 7 to 8 weeks.

This report documents the ease of the nonsuture technique and the high incidence of wide patency in a 1- to 2-year follow-up evaluation by anatomic observations and in vivo flow studies.

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METHOD

Thirty-three dogs, 21 mongrels and 12 greyhounds (19.9 to 36.4 kilograms average 25.2 kilograms, and 20.9 to 28.6 kilograms, average 25.5 kilograms, respectively), underwent nonsuture anastomosis of the left internal mammary artery to the circumflex branch of the left coronary artery near its origin. The anastomosis was performed under Nembutal anesthesia through a fourth left intercostal space thoracotomy, with respiration supported by a positive-pressure respirator using room air. The proximal circumflex artery was dissected from the epicardial fat for a distance of 2 cm., usually with sacrificing of 1 or 2 small descending branches. The left internal mammary artery was then dissected free and its branches tied, clamped at its origin, and transected at the level of the fourth intercostal space. Cuffs available for the anastomoses were of either nylon (for 18 dogs) or stainless steel (for 15 dogs). The cuffs had been previously machined to 4 mm. lengths, 0.15 mm. in wall thickness, and in an appropriate range of internal diameter (2.4 to 3.7 mm., average 2.9 mm.). The appropriate size for the prosthesis was chosen by micrometer measurement of both the internal mammary and circumflex arteries prior to clamping. The size selected was just larger than the external diameter of the internal mammary artery (2.0 to

3.75 mm., average 2.75 mm.) and slightly smaller than the external diameter of the circumflex artery (2.0 to 4.0 mm., average 3.2 mm.). Such snug fits did not significantly interfere with the technique. In 1 animal the diameter of the internal mammary artery at the fourth intercostal space was too small; hence, the subclavian artery was transected beyond the origin of the internal mammary artery and turned down, so that a more proximal portion of the internal mammary artery of larger diameter could be used for the anastomosis. The cut end of the internal mammary artery was then carefully everted over the cuff, with avoidance of intimal trauma, and secured with 2 circumferential fine silk ligatures (Fig. 1, *A*). The circumflex coronary artery was ligated close to its origin and the cuffed internal mammary artery was inserted into the circumflex artery distal to the ligature through an inverted T incision (Fig. 1, *B* and *C*). Flow was reestablished promptly by removal of the clamp on the internal mammary artery, which was then secured within the circumflex artery by 1 or 2 circumferential ligatures about the coronary artery, over the cuff (Fig. 1, *D*). The circumflex artery was then completely transected between the ligature and the anastomosis.

Electrocardiograms were secured preoperatively, continuously during the time of circumflex arterial occlusion, and at selected intervals postoperatively. Arteriography was performed by retrograde femoral arterial catheterization of the aorta in 8 dogs, 13 days to 5 months postoperatively. A single roentgenogram was obtained at the end of a 10 ml. hand injection of 70 per cent Diodrast near or into the ostium of the left subclavian artery.

The animals were allowed to survive 12 to 24 months before additional studies or

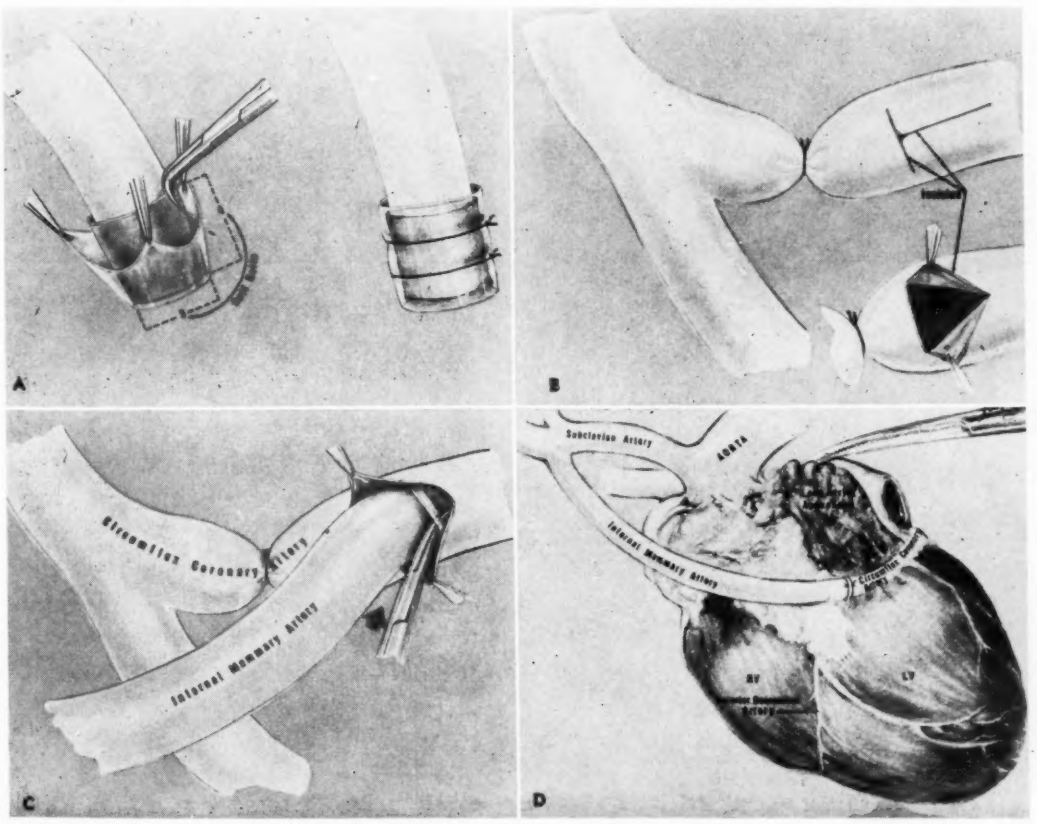

Fig. 1. Technique of anastomosis with tooled nylon or stainless steel prosthesis.

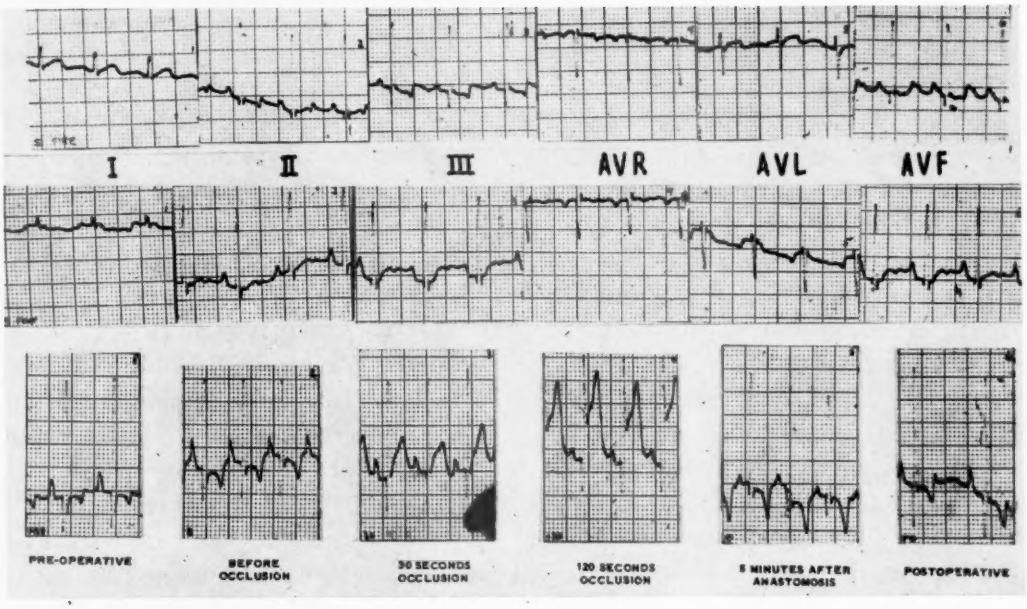

Fig. 2. Electrocardiogram before (top) and after (middle) anastomosis. Lead aV_F (bottom) recorded continuously during period of anastomosis demonstrates marked current of injury pattern during acute occlusion of circumflex artery with prompt recovery after restoration of coronary flow.

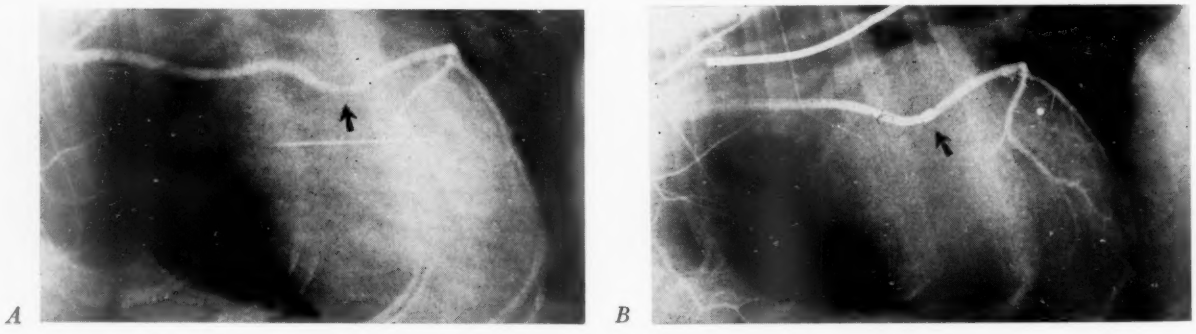

Fig. 3. Arteriogram taken 2 weeks after anastomosis with a nylon prosthesis *(A)*, and 7 weeks after anastomosis with a stainless steel prosthesis *(B)*.

sacrifice. In vivo coronary flow studies were conducted in 14 dogs by the placement of a Kolin-type noncannulating electromagnetic flowmeter probe[7] proximal to the anastomosis on the left internal mammary artery. In 10 dogs, prior to sacrifice for anatomic studies and 13 to 17 months after construction of the anastomosis, coronary flow and central aortic pressure were recorded under Nembutal anesthesia with the chest open. Zero flow reference was repeatedly obtained by briefly clamping just beyond the probe. In the remaining 4 dogs, flow was measured daily over 2 to 4 weeks with the chest closed and the dogs in an unanesthetized state. In one of these the probe was placed about the internal mammary artery at the time of anastomosis and in the remaining 3 at the time of repeat thoracotomy 17 to 24 months after construction of the original anastomosis. Zero flow reference was secured by use of a special circumferential, nylon encapsulated, inflatable, rubber balloon placed around the internal mammary artery just distal to the flowmeter probe. This could be inflated with a known

volume of fluid through a tube led externally with the flowmeter wires through a posterolateral stab wound. Postmortem studies were carried out on all dogs after death or sacrifice. The anastomoses were opened longitudinally with sharp scissors; the prosthetic cuffs were easily cut and could be flattened and slipped out of the encapsulated plane. Longitudinal sections were taken through the anastomosis for hematoxylin and eosin and elastica stains.

RESULTS

Occlusion time. Construction of the anastomosis, timed from the moment of ligation of the proximal circumflex artery until flow was restored, required from 54 to 183 seconds (average 94 seconds) in 28 dogs. Ventricular fibrillation was not encountered in this group. In 5 dogs the occlusion time was 151 to 480 seconds (average 261 seconds) and in each ventricular fibrillation occurred. Ventricular fibrillation was the direct result of technical difficulties which interfered with the prompt performance of the anastomosis in 4 dogs, and occurred after onset of perfusion after 151 seconds in 1 dog. Successful defibrillation was performed in 2 animals and 1 survived with a widely patent anastomosis for 13 months.

Electrocardiogram. Continuous recording of lead aV_F during the period of circumflex coronary arterial occlusion demonstrated various degrees of S-T and T changes consistent with acute myocardial hypoxia. These changes reverted promptly to preoperative patterns upon restoration of coronary circulation. Postoperative records of the dogs with patent anastomoses did not vary significantly from the preoperative tracings (Fig. 2).

Arteriography. Opacification of the internal mammary and circumflex arteries was accomplished in 8 animals. In 6, in which a radiolucent nylon cuff had been used, an excellent lumen diameter was visualized at the site of the anastomosis. Good filling of the distal circumflex artery was visualized in all 8 dogs (Fig. 3).

Survivals. Three dogs died at operation because of technical problems and unsuccessful anastomoses. Three died 3 to 36 hours postoperatively, 1 from separation of the anastomosis, 1 from buckling of a thin nylon cuff with thrombosis of the lumen, and 1 secondary to extensive subepicardial infarction due to repeated defibrillation. Twenty-seven dogs survived operation and the immediate postoperative period. Three of these died, 2, 3, and 13 days postoperatively from thrombosis at the anastomotic site (Table I).

Twenty-four dogs were long-term survivors with widely patent anastomoses. Three died of noncardiac causes, 7 and 10 weeks and 13 months postoperatively. Two were sacrificed in flowmeter studies 1 and 8 months postoperatively; the remaining 19 animals were sacrificed 12 to 24 months, average 15½ months, postoperatively.

Postmortem studies. The longitudinally opened anastomoses disclosed no thrombi or compromise of the lumen in the 24 long-term survivors (Fig. 4). The anastomoses were widely patent with occasional mild wrinkling of the intima within the prosthetic cuff which did not materially alter the lumen size. The distal coronary bed was patent in all. Small patchy areas of myo-

Fig. 4. Gross specimen from greyhound which demonstrates wide patency of anastomosis after 14 months. The left atrial appendage is retracted off the anastomosis site. The internal mammary artery has been opened longitudinally through the anastomosis into the circumflex artery.

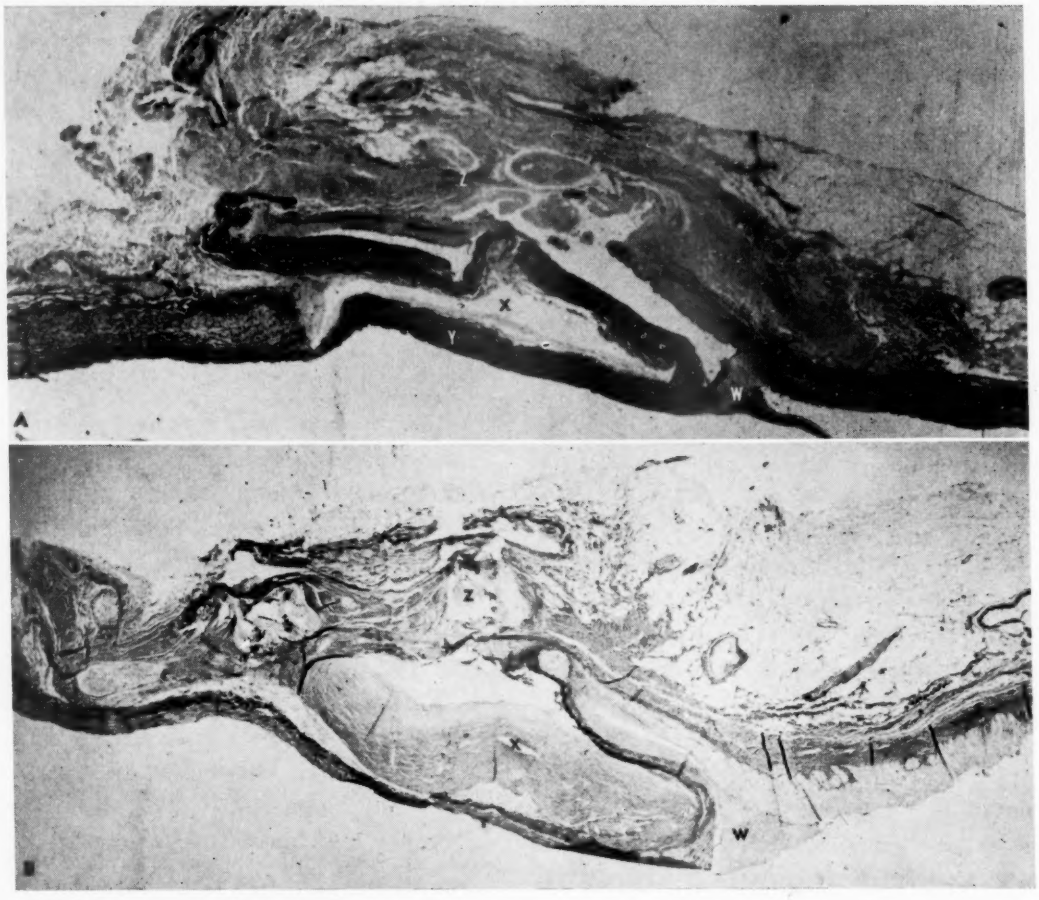

Fig. 5. Longitudinal sections through the internal mammary (left)–circumflex coronary (right) anastomosis. *A,* Nylon prostheses 13 months postoperatively. *B,* Stainless steel prosthesis 13½ months postoperatively. The vessel lumen is at the bottom. The fibrocollagenous wedge at the juncture of the 2 vessels is noted (*W*). Heavy fibrous reaction is seen at the site of the prosthesis (*X*) when stainless steel is used. There is thinning and compression of the elastic lamellae beneath the prosthesis (*Y*), and perivascular foreign-body reaction (*Z*) due to the circumferential ligatures. (Elastic stain; ×23; reduced 4/9.)

cardial fibrosis were seen in a few dogs in the area of distribution of the small coronary branches sacrificed in the circumflex artery dissection.

Histologic studies. The intima was well preserved in all chronic survivors, although in some, thinning and compression of the elastic lamellae of the internal mammary artery due to compression of an expansile vessel against the prosthesis was noted. Varying degrees of proliferative fibrous reaction were present about the prosthesis and were more intense in animals in which a steel cuff had been used. At the junction of the internal mammary and circumflex arteries, a small fibrocollagenous wedge was noted, probably secondary to organization of localized fibrin deposits at the site of juncture of the 2 vessels. This did not project into the lumen, but apparently served to smooth out the lumen at the anastomosis. This location appeared to be the site of intravascular thrombosis in the 3 animals which died of occlusion of the anastomosis in the first 2 weeks postoperatively. In one of these, a distinct circumferential thrombus formation which occluded the lumen, with origin at this junction, could be noted. Sections of the myocardium disclosed no abnormalities (Fig. 5).

Table I. *Survival and patency data (33 dogs)*

Technical errors	
Died at operation (ventricular fibrillation)	3
Died 3 to 36 hr. postoperatively	3
Survived operation and immediate postoperative period	27
Died of thrombosis at anastomosis 2, 3, and 13 days postoperatively	3
Long-term survivors with patent anastomoses	24
Died of other causes 7 and 10 wk. and 13 mo. postoperatively	3
Sacrificed in flowmeter studies, 1 and 8 mo. postoperatively	2
Sacrificed 12 to 24 mo. postoperatively (average 15½ mo.)	19
Anastomoses patent by	
Gross observation	24
Angiography	8
Electromagnetic flowmeter data	14

Coronary flow studies. Measurements of blood flow through the anastomosis were obtained in 14 dogs (Fig. 6). Flows of 31 to 118 ml. per minute (average 68 ml. per minute) were recorded at mean aortic pressures of 100 to 150 mm. Hg. Blood flow was again measured during the period of reactive hyperemia which immediately followed brief (8 to 15 seconds) total occlusion of the internal mammary artery. During this period peak flows of 69 to 259 ml. per minute (average 154 ml. per minute) were recorded at essentially unchanged aortic blood pressures. This represented a 1.9- to 5-fold (average 2.3-fold) increase over the control measurements. Long-term flow measurements, carried out in 4 unanesthetized animals with the chest closed were terminated in 2 to 4 weeks because of a gradual decrease to cessation of measured coronary flow. Autopsy showed a thickening of the vessel wall and thrombosis under the occlusive rubber cuff which resulted in vessel closure. Flow determination in the short- and long-term observations are tabulated in Table II.

DISCUSSION

For a number of years surgical efforts have been directed at improving small-vessel anastomoses, especially in the coronary system, where other methods of myocardial revascularization are indirect. Systemic-to-coronary artery suture anastomoses have met with restricted success because of the significant surgical mortality rate and the high incidence of thrombotic occlusion secondary to intimal trauma. Current microsuture techniques may circumvent some of these difficulties by making possible more exacting anastomoses and consequent reduction of intimal trauma. Nonetheless, such methods remain tedious and time-consuming and, hence, continue to require a concomitant supply of oxygen-

Table II. *Circumflex coronary artery flow data*

Dog. No.	*Flow control*	*Hyperemia, peak (ml./min.)*	*Mean blood pressure*	*Heart weight (Gm.)*
Short-term, chest open, dog anesthetized				
4	105	–	100	369
16	34	105	110	230
21	87	259	125	290
22	57	133	–	–
23	84	258	125	232
24	35	129	100	200
25	118	256	135	250
26	100	191	125	217
28	31	160	150	281
30	55	136	120	328
Long-term, chest closed, dog unanesthetized				
1	42-62	92-124	–	–
13	69	150	–	285
31	30-41	69-97	–	–
33	52-76	200-232	–	–

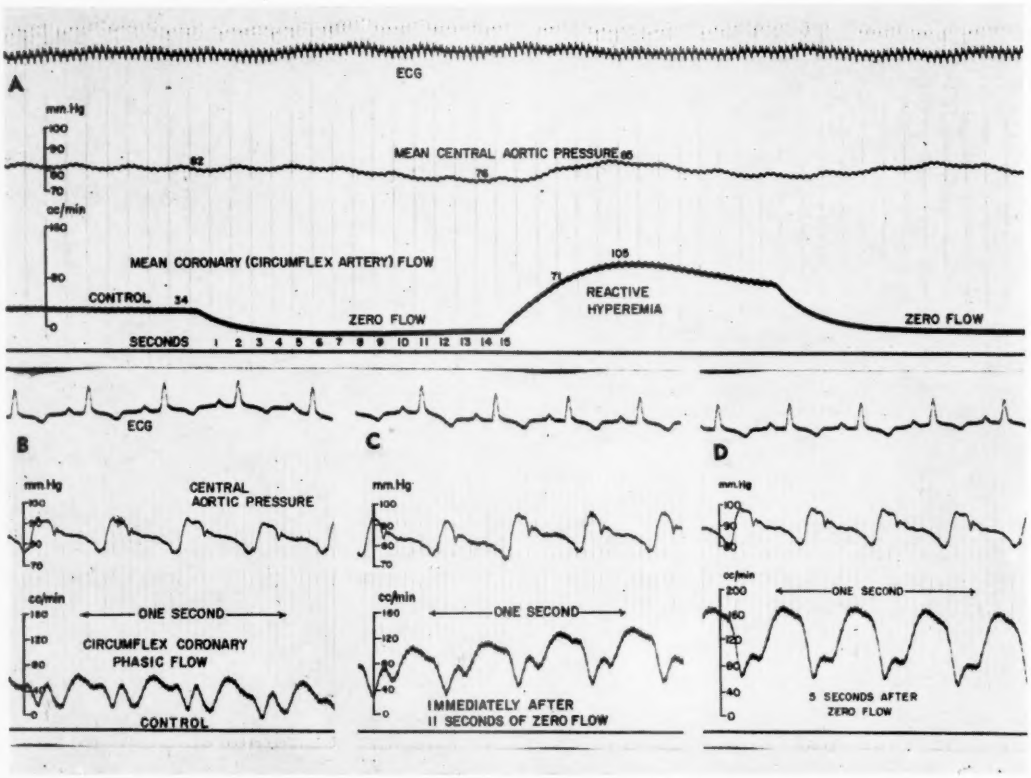

Fig. 6. Open-chest coronary flow measurements with an electromagnetic flowmeter 13 months after anastomosis. The perfused myocardium (Evans blue dye) weighed 55 grams. In *A*, marked reactive hyperemia is seen after brief total occlusion (mean flow). *B*, *C*, and *D* demonstrate phasic coronary flow before, immediately after, and 5 seconds after brief occlusion. *D* is during peak reactive hyperemia. The time lines are at 0.04 second intervals. Note the major flow increase during both systole and diastole. The phasic coronary flow curve is delayed slightly because of the considerably lengthened blood flow path through the internal mammary anastomosis.

ated blood to the myocardium or depression of myocardial function. A nonsuture technique is reported which combines ease and rapidity of performance and thus eliminates the need for myocardial perfusion. This, together with minimal vascular handling and intimal trauma, greatly reduces the incidence of intraluminal thrombosis. The presence of an inert prosthesis for up to a 2-year period, with surrounding mild proliferative changes, has not appeared to compromise the lumen orifice or flow through the anastomosis. In this series thrombotic occlusions at the site of the anastomosis have not occurred after the first 13 postoperative days. Widely patent anastomoses were demonstrated grossly in all long-term survivors, and electromagnetic flow studies supported the functional patency in 14 unselected animals prior to sacrifice up to 2 years after operation. Since compensatory dilatation of the coronary arteriolar bed is the first response to proximal occlusive changes in the major coronary system, the observation of striking augmentation of flow through the anastomosis during the period of reactive hyperemia is interpreted as further evidence of persistence of peripheral arteriolar tone and of wide functional patency at the anastomoses.

SUMMARY

Nonsuture anastomosis of the internal mammary and circumflex coronary arteries is reported with observations for up to 2 years postoperatively. Twenty-four dogs, 90

per cent of those surviving operation and the immediate postoperative period, had widely patent anastomoses. Patency was confirmed by direct measurement of flow through the anastomosis by means of an electromagnetic flowmeter, by observation of significant flow augmentation during the period of reactive hyperemia after brief total occlusion, and by gross and histologic studies.

ADDENDUM

Since preparation of this manuscript, Goetz and associates (Internal mammary–coronary anastomosis. A non-suture method employing tantalum rings, J. Thoracic Surg. 41: 378, 1961) have reported success with a similar technique. They report patency in 8 of 9 anastomoses performed in 7 dogs. Of these 7 animals, 3 were sacrificed after 6 months and the remaining 4 were alive 7 to 20 months after operation.

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Reappraisal

Conducted by CLARENCE DENNIS, M.D., AND ALFRED HURWITZ, M.D.

Cancer risk in ulcerative colitis: formidability per patient-year of late disease

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In 1952 we reported a series of 267 patients with idiopathic ulcerative colitis studied at the University of Minnesota Hospitals and at the Minneapolis General Hospital.[4] In this series of patients, there were 16 who up to that time had developed cancer in the large bowel, a patient incidence of 6 per cent. Four of these patients had been found to have a segmental area of colitis proximal to an obstructing carcinoma. A fifth had developed carcinoma in the stalk of one of several large polyps in the colon 3 years after the onset of colitis. The presence of colitis was regarded as incidental in these 5 cases. With the elimination of these 5 cancers, the incidence of carcinoma was 4.2 per cent. In view of the rather widely divergent opinions to be found in the literature concerning the risk of cancer development in colitis, and since we have found no other reports of an over-all incidence as high as ours is at present, an additional report on this group of patients appears appropriate.

We have endeavored to bring the follow-up on this group of patients up to date by interviews and examinations during the summer of 1957 and by correspondence since that time. Addition of 3 patients overlooked in the original study brings the total to 270. One of these, however, we have omitted from the present study because follow-up was inadequate, and we believed she would not have been found had she not died of carcinoma of the colon while under the care of a physician who knew of our interest in the problem.

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Our over-all data concerning cancer development in these 269 patients are presented in Table I. The over-all patient incidence of cancer in this study was 7.4 per cent. One patient developed rectal cancer late after colectomy for colic cancer; the cancer incidence is therefore slightly higher than the patient incidence—7.8 per cent. If one excludes the 5 cases in which the tumors are considered not to be secondary to colitis, these figures are 5.7 per cent and 6.0 per cent.

Two factors had led us to re-evaluate our data rather than simply to present the figures of Table I. The first was the possibility of inaccuracy of the estimate of incidence of cancer in this group secondary to inclusion of patients who first came for attention because of appearance of cancer of the large bowel, rather than because of the presence of idiopathic ulcerative colitis without such complication. Our second reason was our failure to find in the literature any data concerning the annual cancer risk of the patient with late colitis who continues to carry all or part of the large intestine.

Four patients in this review had already developed cancer as a complication of colitis at the time they were first seen by us. These 4 patients had histories of colitis for 22 years, 15 years, 12 years, and 30 years, respectively, before they were seen. How to

correct our figures for this distortion is not entirely clear.

It appeared more useful to determine the time of earliest appearance of cancer in this group of patients, and the annual incidence of this complication after that time. In regard to time of onset of cancer, the series contains no instance of carcinoma secondary to colitis which appeared within 10 years of the onset of colitis.

In the determination of the actual cancer incidence in patients who carried the large intestine or part of it for longer than 10 years after the onset of colitis, 2 criteria for inclusion of cases were observed. We confined our attention (1) to patients who were under observation at the University of Minnesota Hospitals or Minneapolis General Hospital at the time of the tenth anniversary of the onset of colitis, but who had no evidence of cancer of the large bowel at that time, and (2) to patients who first came to one of these clinics after the tenth anniversary of the onset of colitis without evidence of cancer at that first visit. The period of observation might be terminated by (1) death of the patient, (2) loss of the patient to follow-up, (3) development of cancer in the large bowel, or (4) removal of the colon or rectum, or both, as the case might be.

Findings are shown in Tables II and III.

COMMENT

The figure of greatest note is the development of some 4 cancers per 100 patient-years of observation in colitis in which the whole large bowel was retained after the tenth anniversary of the onset of disease. This figure (4 per cent per year) is higher than the risk of proctocolectomy and ileostomy in the present series (under 2 per cent).[4] The risk of rectal cancer appears to be smaller than that of colon cancer, but even so it is consequential, especially in view of absence of death in the proctectomies performed after colectomy had been performed[4] and in view of retention of sexual function in 100 per cent of patients in whom the conservative proctectomy indicated in the absence of carcinoma was performed.

In our previous paper, we indicated that the risk of ileoproctostomy after colectomy is greater than the risk of proctocolectomy with ileostomy, and that the likelihood of satisfactory function after such anastomoses is less than 50 per cent. The fact that 4 of the 5 rectal cancers which we observed to develop led rapidly to the death of the patient suggests to us that the 1 per cent per annum incidence and the high fatality of cancer when it does develop in the rectum in ulcerative colitis should indicate removal of the rectum in the first place.

The decision as to what duration of chronic idiopathic ulcerative colitis should dictate proctocolectomy is difficult to make.

Table I. *Cancers associated with idiopathic ulcerative colitis*

	No.
Ulcerative colitis cases observed	270
Patients with associated large bowel cancer	21
Case omitted for failure of follow-up	1
Cancer of rectum over 1 year after colectomy for colitis with obstructing cancer of cecum	1
Total primary cancers considered	21
Cancers primary in colon	16
Cancers primary in rectum	
Precolectomy	2
Postcolectomy	3
Cancers present upon first examination of patient	4
Cancers not secondary to colitis (?)	5
Surviving cancer patients*	2

*Both survivors refused proctocolectomy which had been advised years before the diagnosis of cancer as a complication had been established. One had an obstructing cancer of the cecum treated by emergency colectomy, and then refused proctectomy until symptoms of a cancer there had appeared. She is well more than 5 years after proctectomy. The other has survived more than 2 years after removal of colon in which cancer was found.

Table II. *Cancers seen to develop in patients under observation for idiopathic ulcerative colitis*

Under 10 years of ulcerative colitis	0 cases
Over 10 years of ulcerative colitis	12 cases
Average duration of colitis	16 yr.
Average patient age at diagnosis of cancer	35 yr.

Table III. *Large bowel carcinomas which appeared in patients under observation for idiopathic ulcerative colitis*

	Colon or rectum without colectomy or proctectomy	*Colon alone*	*Rectum, combined (pre- and post-colectomy)*	*Rectum after colectomy*
No. of patients followed	60	60	97	54
No. of cancers to appear	9	7	5	3*
Patient incidence of cancer	15%	11.7%	5.2%	5.6%
Patient-years of observation	218	216	443	229
Mean duration of observation	3.6 yr.	3.6 yr.	4.6 yr.	4.2 yr.
Cancers per patient-year	4.1%	3.2%	1.1%	1.3%

*Two of the rectal cancers to appear after colectomy were diagnosed in patients who had undergone ileoproctostomy (out of a total of 42 ileoproctostomies).

Several authors have presented reports of cases in which carcinomatous degeneration secondary to ulcerative colitis developed 3 to 4 years after onset of the colitis.[1, 2, 8] The rarity of such reports suggests that the risk of this complication this early in the course of idiopathic ulcerative colitis is much less than after the 10 years. As in 1952, our present data suggest that the rate of cancer occurrence rises as the years pass after the tenth anniversary of the onset of colitis. The numbers are too small for conclusive decision on this point, however. Certainly, at 10 years after onset of colitis, the risk of cancer development is already very high.[3, 5-7] It has been our policy to recommend proctocolectomy in unsatisfactorily controlled idiopathic ulcerative colitis of 3 or 4 years' duration or in the presence of advancing fibrosis which might mask the diagnosis of carcinoma by roentgen study.

A most dangerous pitfall is to judge the status of the colitis by the general apparent well-being of the patient. A healthy-looking patient all too often harbors a colon and rectum in which advanced proctocolitis or perhaps even cancer is present. Critical proctosigmoidoscopy and barium enema roentgen examination at regular intervals (no more than 6 months) are essential to conscientious care of colitic patients who retain the colon and rectum or the rectum alone. Seventeen years ago, one of the present series, a robust girl of 19 years, asked and was permitted to attend a high school dance within 6 weeks of her death from unsuspected carcinomatosis arising in the colon. Known colitis had been present for 14 years. In the light of present knowledge, this sort of tragedy is clearly avoidable.

The deplorable survival rates after vigorous therapy for cancers developing in patients under observation for idiopathic ulcerative colitis, in addition to the high incidence of cancer in colitis, dictate that our only effective therapy of this common complication is prophylactic removal of the large bowel in all cases in which colitic deformity persists beyond 3 to 4 years.

CONCLUSIONS

1. The group of 267 patients with idiopathic ulcerative colitis reported in 1952 to have 16 large bowel cancers has been followed further. Four more cancers have been found. A fifth is omitted for statistical reasons. The over-all patient incidence of cancer complicating colitis is 7.4 per cent in this series.

2. Prolonged observation is reported in a series of 98 patients with idiopathic ulcerative colitis beyond the tenth anniversary of onset of the disease in whom there was no evidence of cancer at the start of the observation period.

3. In 60 of these patients with colons and rectums in situ and followed for a total of 218 patient-years, the incidence of cancer development was 4 per cent per year.

4. In 54 of these patients, colectomies had been performed, but the rectums had been retained. In a total of 229 patient-years of

observation, the incidence of cancer development in these rectums was 1 per cent per year.

5. We can report only 1 long-term and 1 short-term survival in the 15 patients with large bowel cancer secondary to ulcerative colitis.

6. After the tenth anniversary of the onset of disease, the patient with idiopathic ulcerative colitis bears an annual risk of cancer development of a magnitude approximately equal to that of proctocolectomy in good hands, quite apart from the other hazards of the late disease.[4] After this procedure, his outlook for survival is equivalent to that of persons of similar age without history of colitis.[4]

7. Competent care of the patient with idiopathic ulcerative colitis requires examination of the large intestine at least every 6 months. The risk of cancer is so high that removal of the colon, and preferably of the rectum also, should be performed if the bowel fails to revert to virtually normal flexibility and mucosal appearance within 3 to 4 years of onset of colitis.

The authors wish to express their thanks to Dr. O. H. Wangensteen, Surgeon-in-Chief, to Mr. Ray Amberg, Superintendent, of the University of Minnesota Hospitals, and to Dr. Claude Hitchcock, Chief of Surgery, Minneapolis General Hospital, for permission and facilities to follow and to re-examine these patients.

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Antyllus, the elusive surgical genius of antiquity: an analysis of his writings

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In the study of historical figures there is occasionally encountered an enigma—an author who is afforded a great reputation, but about whom, personally, nothing is known. Just such a phantom is Antyllus, a physician and surgeon of the Graeco-Roman period whose medical and surgical writings have been known and acclaimed in almost every age. The presentation of his surgical writings will be the primary purpose of this paper. However, in spite of his wide popularity in the medical world, few facts have been uncovered concerning his life. Even the exact time in which he lived has been a subject of controversy among medical historians, although most authorities believe it to have been the early part of the second century.

Despite the dearth of information about his life, some insight into his personality can be gleaned from his writings. Although these are far from autobiographic, they do convey a considered judgment and boldness tempered with caution—principles which surely guided his life as well as his practice of medicine. Additional light may be shed on this evasive shade, Antyllus, by a consideration of the wealth of information available on the study and practice of medicine in his day. It is hoped that the speculations made from a study of this material may coincide with the unrevealed career of Antyllus.

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ANTYLLUS AND THE PRACTICE OF MEDICINE DURING THE ROMAN EMPIRE

The first physicians in Rome were Greek slaves, and medicine was held by the Romans to be an ignoble profession to which no free man would devote himself. Gradually, however, a change occurred principally as a result of the increased respectability and intelligence of the more recent immigrant Greek physicians. In 46 B.C. Caesar granted citizenship to physicians, and by the time of the Roman emperors Trajan (A.D. 98 to 117) and Hadrian (A.D. 117 to 138), when Antyllus probably lived, the physicians had achieved considerable social stature. They were well paid and enjoyed all the privileges and prerogatives of the Roman citizenry. They were a class unto themselves, protected by law, and accorded special guarantees by the state. If Antyllus had been as successful a physician as the character of his works leads us to assume, he undoubtedly would also have been a recipient of these special privileges and thus his life would probably have been a comfortable one.

To understand the character and knowledge of the physicians of Antyllus' day, it must be understood that long before the separation of medicine into the many specialties with which we are acquainted, good medicine was usually practiced by men with broad interests and a liberal education in the art of healing. Medicine, surgery, dietetics, pharmacy, and hygiene were indis-

pensable parts of the whole of the practice of medicine. Men trained in all these fields abounded in the early Christian Era and are typified by the famous Galen (A.D. 133 to 200) and many others whose works are preserved in the comprehensive compilations of Oribasius (A.D. 325 to 403) and Paulus of Aegina (seventh century), 2 Byzantine physicians whose main contribution to medicine was the preservation of previous medical thought.

If the methods of treatment of these Graeco-Roman physicians sometimes seem strange and their theories of disease sometimes fallacious, it must not be ascribed to lack of intelligence or ability. Their capacity to practice medicine, so meagerly equipped with the scientific tools that appear indispensable today, is a tribute to their ability. Indeed, when one considers the lack of technical knowledge and the general level of scientific advancement of their time, these men stand out as amazingly resourceful and intelligent.

How most of these renowned physicians of antiquity obtained their training is well known, but so far as Antyllus is concerned, we can only speculate. Because of the considerable influence of previous thought discernible in his writings and because of the impact of his works on subsequent authors, it is possible that he resided in Rome, or at least lived part of his life there. He probably attended one of the many medical schools on the shores of the Mediterranean, such as Alexandria, Smyrna, Rome, Athens, or Marseilles, although definitive proof of this has yet to be discovered. Perhaps he obtained his wide experience during a tenure in one of the larger cities as public physician—an appointment which would have been earned by him for his unusual ability and great resourcefulness—or perhaps as a court physician. He may have obtained his surgical training while serving as an army physician or even as doctor for the gladiators at a gymnasium or at the arena. The latter position offered some of the best opportunities for the practice of surgical techniques and the observation of human anatomy during a time when human dissection was not practiced.

Another subject for speculation is Antyllus' nationality. It is assumed that he was a Greek, for his writings are composed in that language. Almost all of the physicians of the Roman period, including Galen, were Greek and, indeed, in the history of medicine, this period is more rightly called the Graeco-Roman period because all of medicine had its roots in Greek medical thought. Despite Rome's many contributions in other fields such as architecture, warfare, law, and politics, its medicine consisted almost exclusively of the amplification and promulgation of previous Greek thought by foreigners, mainly Greeks.

During the Graeco-Roman period there were several schools of medical philosophy, each of which held to a different idea of the causation of disease. This accounts for the diversity of treatments for the same disease which were recommended by the adherents of the several schools. By Antyllus' time there had developed an eclectic medical philosophy which drew from the other schools only principles and ideas which seemed effective and useful. Most of the unpractical theory was discarded. From the available evidence it can be concluded that Antyllus almost certainly belonged to this Eclectic School and sagaciously blended the theories of the other schools into a reasonable and practical whole. All of the theories and principles which were then so heatedly debated have long since been discarded because science has answered the questions upon which the ancients were forced to theorize. In the context of the times, however, physicians who practiced medicine in an eclectic fashion were the most progressive and contributed the most to the evolution of medical thought.

Because of the intelligence and insight of Antyllus and men like him, it was in surgery, rather than in other fields, that great progress was made during the Graeco-Roman period. Beyond this early surgical art, major improvements and innovations were not made until the advent of anesthesia and antisep-

sis. In fact, for more than a thousand years after the decline of Graeco-Roman medicine, much of the practice of surgery fell into disuse and ill-repute and was far below the level at which it was practiced by the physicians of the Roman Empire. One of the chief reasons for this high level of surgical practice in antiquity, with its more rational approach to treatment, is that in surgery, in contrast to medicine, the theories which contributed so much to deflecting medicine from paths of progress, did not play so large a part. The more purely technical nature of surgery with its clearly obvious results created a situation in which the usefulness and practicality of a procedure were highly prized and more easily obtained. Antyllus and the other physicians of the times, who adhered to a similar philosophy, were free to a greater extent to depend upon and learn from observation and study. They had to contend less with rigorously formal thought while attempting to analyze and categorize their ideas and findings and put them into use. It is this freedom of thought, as well as a large measure of skill, which made Antyllus' surgical works of such value to later physicians and created for him a reputation as a resourceful and experienced surgeon.

THOUGHTS ON THE SURGICAL WRITINGS OF ANTYLLUS

Almost as elusive as Antyllus, the man, is Antyllus, the surgical author. So far as is known, none of his original writings has survived. It is fairly certain that the Byzantine encyclopedists, Oribasius (fourth century) and Paulus of Aegina (seventh century), and perhaps even the Arabian physicians, Rhazes (tenth century) and Avicenna (eleventh century), had direct access to his writings. After this time, however, no author quotes directly from any of Antyllus' works. All subsequent commentators were forced to use the writings of these four as the main sources for the study of Antyllus' thought.

The most inclusive source is the compilation by Oribasius. The translation by Bussemaker and Daremberg[3] of the Greek text of Oribasius was used for this paper, supplemented by selected parts of the writings of Antyllus found in Paulus of Aegina, as translated by Francis Adams.[5]

From the fragments available it seems that Antyllus wrote at least 2 major treatises, the first mainly concerned with medicine and the second with surgery; and although Antyllus is best known for his surgical works, his writing on other aspects of medicine show the same careful approach based on wide experience that is apparent from the surgical chapters. It is hoped that a brief outline of some of his chapters on surgery will be sufficient to demonstrate the ability and intelligence of this sage clinician. The medical works of Antyllus have been discussed in a previous paper[2] and with the exception of several methods of treatment which are actually surgical in nature, such as venesection, they will not be mentioned. Unfortunately, it would require a full reproduction of Antyllus' surgical works to do complete justice to the thoroughness and remarkable clarity with which he treats his subjects. Every page manifests the wide experience and cautious but sure judgment which served as the basis for Sir William Osler's designation of Antyllus as one of the most daring and accomplished surgeons of all time.

Most of the physicians of Antyllus' time were not specialists; they wrote on all medical subjects. Antyllus' writings, by the standards of the time, however, do not appear to be complete and by comparing them with works of other physicians of his time which have come down to us in more complete and perhaps original form, one notes wide gaps in Antyllus' subject matter. There are no specific sections on gynecology or obstetrics, dermatology, fevers, trauma, anatomy, pathology, physiology, or mental or nervous diseases, although some of these subjects are mentioned in the chapters which treat of other matters. The most likely explanation for this hiatus may be that these works were lost very early and were not included in the fragments which were available to the com-

pilers. In view of the thoroughness with which Antyllus treats his various subjects in the chapters to which we have access, it is unlikely that he neglected to write on the topics mentioned.

ANTYLLUS' SURGERY

For nearly 2 millennia, venesection was one of the most frequent surgical procedures. It is interesting, therefore, to note the extent of thought given and caution exercised in this important operation. Antyllus begins his section on bleeding by venesection with a detailed description of the proper vessels to be opened in different parts of the body. He cautions against thrombosis of a vessel and against incising blindly for a vessel when one is not apparent. He knowingly advises the use of a tourniquet above the site of incision on an extremity because he recognized that "never, on any part, will the band (tourniquet) cause the swelling (distention) of a vessel when it is placed below it."

One entire chapter is devoted to instructions for the actual cutting of the vein. In this he says:

> Bleeding is done, sometimes by thrusting the instrument, sometimes by raising it; by thrusting it, when the vessels are not apparent and raising it when they are very near the surface. It is not necessary to divide the vessel completely, for, in that case, the two ends retract and the blood does not jet out. . . .

Antyllus' knowledge of wound healing, in relation to the type of incision, is demonstrated in the chapters discussing the proper time for the incision and its proper size and form.

> We use the transverse incision, when we have no intention of repeating the bleeding, because once the arm is bent, the wound adheres quickly. The oblique incision is suitable when it is desired to repeat the bleeding, for during the flexion of the arm, the lips of the wound do not touch each other exactly. The straight incision (longitudinal) is also suitable when recourse will be had again to bleeding, not only the same day, but also on the third and fourth, for by flexing the arm, the lips of the wound remain notably separated from one another.

Completing the discussion on the subject of bleeding, Antyllus outlines the maneuvers which were to be resorted to if the flow of blood slowed or halted prematurely. One of the most interesting parts of this chapter is his recommendation that bands be applied to the extremities in the case of fainting due to bleeding. This corresponds to the present-day application of tourniquets to the limbs in cases of peripheral vascular collapse.

Antyllus' treatment of fistulas and abscesses constitutes a fairly complete accounting of these conditions in their various locations and degrees of complication. In fact, little improvement has been made upon his treatment of anal fistulas in the ensuing 18 centuries. His exceedingly bold, though careful, surgical approach to these conditions was mainly that of incision or extirpation in the particular manner required for the various locations. His description of the physical diagnosis and treatment of osteomyelitis is cogent and completely rational in terms of present-day knowledge. He recommends that particular attention be paid to the placement of incisions, following, if possible, the natural skin creases. His boldness was tempered with concern for the esthetic, but he did not lose sight of his real goal:

> . . . in general, it must be known that we teach the shape of the incisions by first taking into account the safety of treatment and the beauty of the form; in fact, when, with our incisions, we imitate the natural lines, we have in view, beauty; but, when we cut perpendicularly, as on the limbs and the posterior region of the neck, we are concerning ourselves with security, standing in dread of being obliged to divide some nerve or some vessel by cutting transversely. It must be recognized that we ought not to attach equal importance to security and beauty, for in all cases, I regard the consideration of the safety to be of particular concern to men.

Antyllus was not completely free of the prejudices and false theorizing of the day, despite the evidence in his works of remarkably acute powers of observation and vast experience. He was still enough of a child of his time to perpetuate the fallacious concept that:

> . . . if it is, on the other hand, an artery which has been eroded (by an abscess), there

will leak out thin, bright red blood, together with whistling and the emergence of a great deal of air.

In another chapter, he advises the surgical removal of steatomas and ganglia. An example of his experience is his caution that one should "always endeavor to remove the capsule (of a steatoma) in its entirety; for, if a part is left, this part is cause of recurrence." Although our understanding of the word steatoma may differ from his, it is obvious that he was dealing with a tumor of the skin, prone to recurrence, in which the only effective cure was complete removal.

Antyllus' best known chapter is his treatment of aneurysms, which was widely used at least until the time of John Hunter (1728 to 1793), the eminent Scottish anatomist and surgeon, who practiced and taught in London. Indeed, James Syme (1799 to 1870), the outstanding surgeon of Edinburgh and father-in-law of Lord Lister, also appears to have followed Antyllus' method. In discussing this famous chapter on aneurysm, Sir William Osler[4] says of Antyllus:

> No other ancient author has anything like the same accuracy of pathologic description, and you may search the surgical literature for centuries before there is found such a gem as the account of his method, still in use, and by which his name has been permanently enshrined.

Antyllus described 2 varieties of aneurysm, those arising from a dilatation of an artery and those arising from escape of blood into surrounding tissues from a traumatized artery—perhaps leading to an ateriovenous fistula. He treated the latter type with percutaneous ligation of the area. His treatment of the former consisted of the placement of ligatures above and below the aneurysm after careful surgical exposure of the vessel, incision, and removal of the contents of the aneurysm with the ligatures left in place and without excision of the aneurysmal section. The caution expressed in his admonition not to remove the dilated section of the vessel demonstrates his experience with the occasional occurrence of loosening and accidental displacement of a ligature from the severed end of an artery, particularly one of good size, subsequent to movement and the arterial pulsations.

On the matter of hydrocephalus, Antyllus seemed to be somewhat less certain. He appears to have confused it with both cephalhematoma and caput succedaneum, for he called them all by the same name. In his description of each, he clearly differentiates them, however. He did recognize that hydrocephalus caused separation of the sutures of the skull.

One of the most daring procedures recommended by Antyllus was laryngotomy or tracheotomy:

> In cases of cynanche we entirely disapprove of this operation, because the incision is utterly unavailing when the whole trachea and the lungs are affected; but in inflammations about the mouth and palate, and in cases of indurated tonsils which obstruct the mouth of the windpipe with the trachea unaffected, it will be proper to have recourse to pharyngotomy (tracheotomy), in order to avoid the risk of suffocation. When, therefore, we engage in the operation, we slit open a part of the arteria aspera (for it is dangerous to divide the whole) below the top of the windpipe, about the third or fourth ring. This is a convenient location, as being free of flesh, and because the vessels are placed at a distance from the part which is divided. Wherefore, bending the patient's head backwards, so as to bring the windpipe better into view, we are to make a transverse incision between two of the rings, so that it may not be the cartilage which is divided, but the membrane connecting the cartilages. If one be more timid in operating, one may first stretch the skin with a hook and divide it, and then, moving the vessels aside, if they come in the way, make the incision.

It is difficult to imagine that such a detailed description of a procedure as this could have been written by a person who had never performed it, but there is, currently, some question as to whether it had ever actually been performed in the Graeco-Roman period. Even Fabricius of Acquapendente (1533 to 1619), the famous Italian surgeon and anatomist and teacher of Harvey, gives an unusually lucid account of the operation, taken in part from Antyllus, but prefaced his description with the remark that he, himself, had never performed it.

Abdominal paracentesis, like tracheotomy, was performed, or at least recommended, before Antyllus' time, but ever mindful of possible difficulties, he contributed several cautions to be kept in mind during the performance of the operation. He suggested that the incision be made on the right side if there is disease of the spleen and on the left if the liver is diseased. He advised that the opening through the peritoneum should be made higher up than that of the skin in order to close more effectively the tract after withdrawal of the trochar. Another recommendation, an excellent example of Antyllus' ability to relate cause and effect, was that the discharge of water should be halted if the pulse should sink during the operation.

Antyllus included urology among his many other interests. He prescribed catheterization for urinary retention, incised for vesical calculi, and attempted correction of hypospadias. One of his omnipresent cautions was that a catheter ought not to be introduced when the retention arose from inflammation at the neck of the bladder. Unfortunately, Antyllus does not mention what should be done.

The other subjects on which Antyllus wrote were colobomas, ectropion, dropsy, hydatids, indurated tonsils, cynanche, gynecomastia, hernia (which he recognized as "a dilatation of the passage which leads from the cavity of the abdomen to the testicle"), and pterygia about the nails. In all of these is reflected the wide experience, interest, and skill of their author. We may assume that the one obvious gap in the wide variety of diseases and problems on which Antyllus touched in his surgical chapters was an intentional one. Knowing full well the penalties in his day which accompanied the performance of such a procedure, Antyllus prudently eschewed entering any of the large body cavities.

In general, most of Antyllus' surgical writings deal with procedures which could be accomplished within the limits imposed by the lack of knowledge of effective anesthetic agents and poor antiseptic technique. These procedures would be either not particularly painful, such as venesection and paracentesis, or would be carried out under conditions of dire necessity when temporizing or refusal might have led to the death of the patient, as in tracheotomy. One facet of the surgical art, which does not seem to fit into this general statement, is plastic surgery. It was usually elective, fairly time-consuming, and designed not for the saving of life or as medical treatment, but specifically for the cosmetic effect or perhaps to increase the usefulness of a part. Yet Antyllus, in the second century, did write on this subject. In fact, Castiglioni,[1] a prominent medical historian of the twentieth century, says that Antyllus'

> . . . indications relative to plastic surgery for defects of the skin and especially the eyelids, forehead, nose, and cheeks are accurate and show that the author was certainly a surgeon of wide experience and unusual ability.

Antyllus also recommended surgical correction of contractures due to scars either from burns or suppuration and had extensive experience with plastic operations on the prepuce and penis.

CONCLUSION

From a brief sampling of his works, it is apparent that Antyllus was a life-long student of his art. Equipped with unusual perspicacity and consummate skill, he approached disease with an open mind and the sole desire to do his best for the patient. His success can be measured by his renown, and his knowledge by the few fragments of his works extant. It is hoped that this résumé of his works, in addition to presenting the thought of this truly skilled physician and surgeon, also provides some insight into the elusive personality of this enigmatic spirit of antiquity.

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ERRATUM

In the paper "Myocardial Response to Prolonged Acute Oligemic Hypotension," by M. G. Weidner, Jr., M.D., Loren Roth, and F. A. Simeone, M.D., in the July, 1961, issue of SURGERY, Figs. 3 and 4 were reversed. The legend appearing under Fig. 3 belongs with Fig. 4 and vice versa.